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Vaccine and epidemiologic studies of Salmonella infections in swine

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Vaccine and epidemiologic studies of *Salmonella* infections in swine

by

David Hamilton Baum

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ABSTRACT

Four vaccine studies were conducted. The first vaccine study determined that pigs vaccinated with SC54™ at one day of age were protected at 35 days of age against infection by *S. choleraesuis*. Vaccine studies two and three determined that SC54™ was not effective in protecting pigs against challenge by *S. typhimurium*. The fourth vaccine study was an investigation of the association of SC54™ use and the reduction of the bacteriologic and serologic prevalence of *Salmonella* in a commercial herd. Results from this vaccine study suggested that vaccination of pigs with SC54™ reduced the bacteriologic prevalence of some *Salmonella* serogroups. The results from this fourth study also suggest that SC54™ may reduce the seroprevalence of *Salmonella* in pigs at the time of slaughter.

Samples of serum and feces from pigs were collected for most of one year from three farms. In addition, mesenteric lymph nodes and meat juice samples were collected from pigs from the same farms at slaughter. The results from these studies suggested that groups of pigs with high levels of *Salmonella* detected by culture also have high levels of antibody to *Salmonella* as measured by the mix-ELISA.

A serologic survey of samples from several farms throughout the Midwest was conducted to detect the presence of antibody to *Salmonella*. A questionnaire was completed by some farms. Pig performance data was made available for some groups of pigs, also. The results from these studies suggested that a decrease in the seroprevalence of *Salmonella* associated with the use of an isolated nursery and with testing of pigs during the first two quarters of the year. It was also shown that there was an association between management factors recorded at the time of pig placement and an increased seroprevalence of *Salmonella*.

Finally, low growth performance based upon the pounds of pork produced (liveweight) per square foot of building per year was associated with groups of pigs that had high levels of *Salmonella.*

In addition, some serum samples were tested for the presence of antibody to *Toxoplasma gondii, Trichinella spiralis*, and *Yersinia enterocolitica* O:3. The prevalence of *T. gondii* and *T. spiralis* antibodies was below 0.01. There was a direct association between the seroprevalence of *Y. enterocolitica* O:3 and the seroprevalence of *Salmonella*.

CHAPTER ONE. INTRODUCTION

In 1994 legislation was drafted by the Congress of the United States that would change the methods for carcass inspection at slaughter facilities. The basis of these changes was founded in the principles of HACCP (Hazard Analysis and Critical Control Point), a systematic approach to food safety. The HACCP system was adopted in 1989 by the National Advisory Committee on Microbiological Criteria for Foods (Corlett and Pierson 1992). This committee described the seven principles of HACCP:

1) Assess hazards and risks associated with growing, harvesting, raw materials and ingredients, processing, manufacturing, distribution, marketing, preparation and consumption of the food.
2) Determine the CCP's required to control the identified hazards. A CCP (critical control point) is any point or procedure in a specific food system where loss of control may result in an unacceptable health risk.
3) Establish the critical limits that must be met at each identified CCP.
4) Establish procedures to monitor each CCP.
5) Establish corrective action to be taken when there is a deviation identified by monitoring a CCP.
6) Establish effect record-keeping systems that document the HACCP plan.
7) Establish procedures for verification that the HACCP system is working correctly.

One feature of the legislation was the routine bacteriologic monitoring of carcasses for the presence of *Salmonella*. Baseline carcass *Salmonella* levels would be determined for

slaughter facilities and used as a guide to determine whether a reduction in the bacteriologic presence of *Salmonella* followed the implementation of HACCP.

***Salmonella* vaccine studies.** In 1992, a live avirulent vaccine was developed for the control of infections in swine caused by *S. choleraesuis* (Kramer, Roof et al. 1992). This vaccine was shown to be effective and safe when administered to swine that were 21 days of age or older. Later studies showed that pigs remain immune to challenge by *S. choleraesuis* up to 20 weeks after vaccination (Roof and Doitchinoff 1995). Pigs that are vaccinated with this product have lower numbers of organs that are colonized by *S. choleraesuis* and shed *S. choleraesuis* for fewer days when compared to nonvaccinated pigs.

Serologic testing for *Salmonella* infection of swine. A serologic test was developed in Denmark (Nielsen, Baggesen et al. 1994) for the purpose of detecting groups of pigs with high prevalence of *Salmonella* infections This test was developed for use in a nationwide program to reduce the bacteriologic levels of *Salmonella* in Danish pork and pork products. Commercial herds that produce more than 100 market animals per year must routinely test randomly-selected pigs at slaughter. The results of serologic testing are used to designate Danish swine herds into one of three levels: 1, 2, or 3. Farms that are designated as level 2 or 3 must submit and implement management changes for the purpose of reducing the prevalence of *Salmonella* infections. In addition, pigs from level 3 farms are slaughtered at special times of the week and in special slaughter facilities. This control program has been associated with a reduction in the *Salmonella* contamination of pork and pork products in Denmark (Nielsen, Bager et al. 1995) .

Purpose of this dissertation. These studies were conducted in order to determine if the live avirulent vaccine reduces *Salmonella* other than *S. choleraesuis*, whether the vaccine can be administered safely at one day of age, whether vaccination at one day of age protects against pigs against challenge with *S. choleraesuis*, and what happens to *Salmonella* populations in a commercial swine farm following the administration of the vaccine.

The Danish mix-ELISA was also evaluated for the purpose of validation in the United States. This validation was conducted by examining the association between the serologic (mix-ELISA) prevalence of *Salmonella* and the bacteriologic prevalence of *Salmonella*. Samples of serum from pigs and pig feces from pen floors were collected from two swine farms for a full year and from one farm for 6 months. These samples were tested to determine if high seroprevalence of *Salmonella* from pigs on the farm is associated with high culture prevalence of *Salmonella* from feces from the farm.

Samples of mesenteric lymph nodes and muscle juice were collected in pairs from swine carcasses at slaughter. These samples were tested to determine if high seroprevalence of *Salmonella* from pigs at the time of slaughter was associated with high culture prevalence of *Salmonella* from mesenteric lymph nodes at the time of slaughter.

Serum samples from 123 groups of pigs from 10 different production sources were tested for the presence of antibodies to *Salmonella*. Each group of pigs was categorized according to the nursery that was used in their production: either an isolated nursery or no isolated nursery. Farm management factors were recorded for some of these groups of pigs in order to determine risk factors that might be associated with increased seroprevalence of *Salmonella*, whether there is an association between *Salmonella* seroprevalence and the

growth of commercial pigs, and whether or not it is possible to monitor certain management practices on a swine farm as possible CCP's for the purpose of reducing the seroprevalence of *Salmonella* in pigs prior to slaughter.

Finally, some groups of pigs were tested for the presence of antibody to *Toxoplasma gondii, Trichinella spiralis*, and *Yersinia enterocolitica* O:3. The results from these tests were used to compare with the seroprevalence of *Salmonella.*

CHAPTER TWO. LITERATURE REVIEW

The role of swine and swine products in the development of infections in humans is of considerable concern throughout the world. Recent changes in the method of animal inspection in slaughter facilities in the United Stated were intended to improve the safety of the meat supply of the United States. The change in inspection methods by the United States' Department of Agriculture, Food Safety and Inspection Service (FSIS), are referred to officially as "Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) Systems; Final Rule" (USDA 1996). These new inspection methods will include visual inspection of animal carcasses and bacteriologic examination of slaughter facilities and carcasses within these facilities. The framework under which these new methods will be employed are referred to as Hazard Analysis and Critical Control Point (HACCP) plans. During the first stages of implementation of this final rule, carcasses will be sampled and tested for the presence of *E. coli*. Levels *E. coli* found in a slaughter facility will be used to establish baseline data about levels of fecal contamination of carcasses. Carcasses will also be tested for the presence of *Salmonella*. Information from *Salmonella* testing will be used to determine baseline levels of *Salmonella* contamination of carcasses and facilities.

It is not the intent of FSIS to use this information to regulate animal production facilities (Billy 1997). However, a critical control point for federally-inspected slaughter facilities will likely be the animals that enter those facilities for slaughter. Therefore, it would be reasonable to believe that the management and procurement personnel of a slaughter facility would want to have information about the animals that it purchases. This information would include data and substantiation of efforts to reduce levels of *Salmonella* in

animals that would be delivered from the farm to the slaughter facility. This change in federal inspection of slaughter facilities, therefore, may affect the routine management strategies used in swine production facilities in the United States.

A purpose of this literature review is to provide information to swine practitioners, pork producers and research scientists regarding what is known about the production of pork with reduced levels of *Salmonella*, *T. gondii*, *T. spiralis*, and *Yersinia enterocolitica* O:3.

Foodborne illnesses of humans

Salmonella infections

In a review of foodborne *Salmonella* infections, Jay (1992) states that, "*Salmonella* food poisoning is caused by the ingestion of foods that contain significant numbers of non-host specific serotypes of *Salmonella*." The significant number of *Salmonella* needed to infected people was reported to vary with the serotype involved but typically requires 10^7 - 10^9 organisms per gram of ingested food. The incubation period is typically 12 to 14 h. Symptoms of *Salmonella* food poisoning include nausea, vomiting, abdominal pain, headache, chills, and diarrhea which can persist for 2 to 3 days. The average mortality rate is 4.1% with differences in mortality associated with age. The mortality rate of infants (less than one year of age) is about 5.8% and the mortality of people over 50 is about 15%. Among the different serotypes, *S. choleraesuis* infection, although rare, has been reported to produce the highest mortality rate of 21% (Jay 1992).

A report by the National *Salmonella* Surveillance System listed the most frequently isolated serotypes of *Salmonella* from cases of human and non-human isolates for the period of January through December, 1992 (Bean and Potter 1994). The five most frequently

isolated serotypes from human cases were *S. typhimurium, S. enteritidis, S. heidelberg, S. hadar,* and *S. newport* while the five most frequently reported serotypes from non-human cases were *S. enteritidis, S. typhimurium, S. heidelberg, S. hadar,* and *S. choleraesuis.* In contrast, a report published by the National Veterinary Services Laboratory (NVSL) indicated that the top 5 isolates from swine were *S. derby, S. choleraesuis, S. typhimurium* (Copenhagen), *S. heidelberg,* and *S. typhimurium* (Ferris and Miller 1996).

***Yersinia enterocolitica* infections**

Infections from *Y. enterocolitica* usually occur following the ingestion of food that had been contaminated with feces containing the organism. Swine appear to be the major source of strains that are pathogenic to humans. The most commonly occurring O groups of *Y. enterocolitica* in human infections are O:3, O:5,27, O:8, and O:9. In the United States, O group O:8 is isolated most commonly (Jay 1992).

The clinical disease syndrome produced by *Y. enterocolitica* is characterized by gastroenteritis. Other syndromes produced by *Y. enterocolitica* include pseudoappendicitis, mesenteric lymphadenitis, terminal ileitis, reactive arthritis, peritonitis, colon and neck abscesses and cholecystitis. Symptoms of gastroenteritis tend to develop several days following ingestion of contaminated foods. Morbidity and mortality rates following *Y. enterocolitica* infection are variable during an outbreak (Jay 1992).

***Toxoplasma* infections**

This obligate intracellular parasite usually does not produce symptoms when people become infected. However, when symptoms do occur they can include fever with a rash, headache, muscle aches and pain and swelling of the lymph nodes. The muscle pain can be

quite severe and last for up to a month or more. The most severe clinical syndromes are produced in immunocompromised people and can produce birth defects in children who are infected *in utero* (Jay 1992).

***Trichinella* infections**

Infections caused by the roundworm *Trichinella spiralis* have declined steadily since 1950 with the exception of occasional epidemics occurring during the decline (Jay 1992). Jay (1992) further describes infection by *T. spiralis* as follows. Infection occurs by the ingestion of encysted meat that has not been properly stored or prepared. The incubation period is dependent on the number of larvae that are ingested. If heavily encysted meat is ingested, the incubation period is only one or two days. In such cases, the migrating trichinae can cause nausea, abdominal pain, diarrhea, and sometimes nausea. If only a few larvae are ingested, the incubation period may be as long as 30 days. Three different outcomes have been described following the ingestion of encysted meat. The three outcomes are dependent on the number of larvae that are deposited in striated muscle. If 10 or fewer larvae are deposited per gram of muscle tissue, there are usually no symptoms. When 100 or more larvae are deposited per gram, symptoms of clinical trichinosis usually develop with very serious clinical consequences developing following the encystment of 1,000 larvae or more per gram of muscle. The universal symptom of trichinosis is muscle pain and is manifested as difficulty in breathing, chewing, and swallowing. About 6 weeks after the initial infection, encystment occurs and is accompanied by tissue pain, swelling, and fever (Jay 1992).

The Centers for Disease Control (CDC), Atlanta, Georgia, reported the results of a Trichinella surveillance study in 1986 (Bailey and Schantz 1986). There were a total 43

cases of trichinosis reported during that year. Twenty-six of those cases were traced to the consumption of pork. Fourteen of the cases traced to the consumption of pork were traced to the consumption of wild boar meat while 6 cases were traced to the consumption of pork sausage that was purchased directly from a farm. Three cases were traced back to the purchase and consumption of commercial pork products. Three cases were traced to pork of unknown or other sources.

Foodborne zoonoses associated with swine

Salmonella infections

Salmonellosis is a collective description of a group of diseases with symptoms which varying from severe enteric fever to mild food poisoning (Lax et al. 1995). All the known serotypes are pathogenic for humans, animals, or both (Lax et al. 1995). Some serotypes of *Salmonella*, such as *S. typhi, S. paratyphi A,* and *S. sendai* appear to be strictly host-adapted and cause disease only in humans (Lax et al. 1995). Other serotypes of *Salmonella*, such as *S. dublin* and *S. choleraesuis* appear to be host-adapted in cattle and swine, respectively, but can cause disease in other hosts. The molecular basis of host-adaptation has not yet been clearly defined. The largest group of serotypes of *Salmonella* are known as non-host adapted serotypes of *Salmonella*. These serotypes cause disease in a wide variety of animals, including human. The most common serotypes of non-host adapted *Salmonella* (such as *S. typhimurium*) are often carried by animals without producing clinical disease other than a transient diarrhea (Lax et al. 1995).

A review of salmonellosis in the United States indicated a moderate increase in the annual incidence in humans and lower animals (Morse 1974). In comparison to reports from

the 1940's and the 1950's this increase was attributed to the following factors: development of better techniques and media for isolation and identification of *Salmonellae*, greater research and diagnostic laboratory activity to detect and confirm cases of salmonellosis, more effective surveillance and reporting efforts by states, CDC, and the USDA epizootics and epidemics of salmonellosis which promote greater public awareness and epidemiologic activity, increased human and animal population densities (for instance, urban ghettos and confinement rearing of livestock), greater mobility of humans and animals that created more opportunity for contact with *Salmonellae*, increased consumption of poultry as well as inadequate cooking, increase in hospital and nursing home populations indicating a shift to the aged and chronically ill, and convenience and mass prepared foods as well as the tendency in an affluent society to eat many meals outside of the home and to experiment with exotic cuisine.

The pathogenesis of *Salmonella* infections has been reviewed (Lax et al. 1995) and will be summarized. Infection by *Salmonella* is primarily fecal-oral although respiratory infection appears to be possible. The dose required to cause infection has been reported to range from 10^3 to 10^{10} organisms, depending on the size (mice vs. cattle) and age of the host. In humans, it is possible for only 10 cells to be infective if the organisms are consumed in chocolate. The high fat content of chocolate protects the organisms from the low pH of the stomach. Entry into tissues probably occurs in the intestines. Once the mucosa has been breached, the organisms appear in the lamina propria and induce a local inflammatory response characterized in part by the infiltration of macrophages. Phagocytosis of organisms occurs and is partially responsible for the dissemination of *Salmonella* to other organ systems

in the body. If an adequate immune response does not develop, *Salmonella* rapidly replicate in the tissues, further stimulating inflammation and necrosis of the tissues. Often during this process, ulceration of the intestinal mucosa occurs with increased shedding of organisms in the feces and the development of diarrhea. Causes of death vary and include severe dehydration if the infection is limited to the intestinal tract or endotoxemia in cases of systemic infection. Severe systemic infections in humans may be exacerbated by factors which affect natural resistance to infection. These factors include rapid stomach emptying, impaired peristalsis, altered gut flora, antibiotic treatment, parasitism, carcinoma, leukemia, sickle cell anemia, and viral or therapeutic immunosuppression.

Human infection from host-adapted *Salmonella* such as *S. dublin* and *S. choleraesuis* are rare. In such instances. clinical disease produced by these serotypes is reported to be quite severe and can include the development of non-enteric infections such as skin abscesses (Lax et al. 1995).

Yersinia infections

Infection of swine by either *Y. enterocolitica* or *Y. pseudotuberculosis* usually produces no clinical syndrome (Taylor 1992). However, these organisms are capable of causing fever, enteritis, and diarrhea in susceptible swine. *Y. enterocolitica* has at least 46 O groups and 5 biotypes of which biotype 2;O:9, biotype 4; O:3 and biotype 1; O:8 are most frequently associated with infections in humans. Fecal contamination of meat from pigs is considered to be the primary source of human *Y. enterocolitica* (Kapperud et al. 1994).

Infections by *Y. enterocolitica* have been reviewed (Taylor 1992) and will be summarized. *Y. enterocolitica* is ubiquitous worldwide and has been shown to be shed in the

feces of infected pigs for up to 30 weeks. It has been reported that *Y. enterocolitica* can be transmitted to humans by flies. The organism has been shown to remain viable in feces for up to 12 weeks and is typically transmitted by the ingestion of fecal contaminated feed or by contaminated debris in facilities. After ingestion, the organisms appear to establish a persistent infection in the tonsil (Kapperud 1994). Occasionally, *Y. enterocolitica* has been found on the surface of slaughtered swine carcasses. Prevention of infection appears to be best accomplished by improving the hygiene of swine facilities and concurrent reduction of populations of flies and rodents.

It has been reported that when pigs are found to be culture-positive for *Y. enterocolitica*, the majority of the finishing pigs of that herd are infected (Kapperud et al. 1994, Kapperud, 1994 #910). Swine have been known to be healthy carriers of serogroups O:3 and O:9. Biovars and phagevars from these serogroups of *Y. enterocolitica* have been with human disease.

An indirect ELISA has been developed for the purpose of screening swine herds for the presence of *Y. enterocolitica* O:3 (Nielsen et al. 1996). Swine herds that had been found to be culture negative for the presence of *Y. enterocolitica* O:3 also were negative for the presence of antibody to *Y. enterocolitica* O:3 (Nielsen et al. 1996). However, swine herds with known infections of *Y. enterocolitica* O:3 were consistently seropositive. A large variation in the culture results was noted among pens in infected pigs from one month to another. It was suggested that the variations in culture results in positive herds is a reflection of the age that pigs are infected with *Y. enterocolitica* O:3. Pigs infected early in life appeared to have cleared the infection from their bodies, yet still retained measurable

antibody to *Y. enterocolitica* O:3. Pigs that would have been infected later in life would be more likely to be detected by culture when samples were collected later in the production cycle. Antibody production was associated with the presence of *Y. enterocolitica* O:3 in palatine tonsils.

A cross-sectional study of 10 swine herds in Denmark was conducted to investigate when *Y. enterocolitica* O:3 might be introduced to pigs in commercial pig production units (Wingstrand and Nielsen 1996). Six sections of each herd were sampled by collecting blood and pooled pen feces. The sections of each herd that were examined included dry sows older than first parity, gilts, suckling pigs, weaners, growers, and finishers. Sows and suckling pigs did not shed detectable levels of *Y. enterocolitica* O:3. Culture results were positive for gilts, weaners, growers, and finishers. The prevalence of *Y. enterocolitica* O:3 increased from weaners to growers to finishers. Seroprevalence followed the same general trend as culture results in pigs: suckling and weaner pigs were seronegative while the seroprevalence increased in the growers and again in the finishers. About one-third of all samples collected from the sows were seropositive. Over one-half of the samples collected from gilts were seropositive. All sow herds were seropositive and 90% of the gilt populations were seropositive. The investigators hypothesized from these findings that pigs are infected from their environment, rather than from their sows and that efforts to control *Y. enterocolitica* O:3 infections should be directed at improving farm hygiene.

***Toxoplasma* infections**

Toxoplasmosis is caused by infection with *Toxoplasma gondii* following the ingestion of sporulated *T. gondii* oocysts or by consuming meat containing tissue cysts (Lindsay et al.

1992). Cats (and other felines) are the only animals that can excrete oocysts in their feces and are believed to be important vectors in the transmission of *T. gondii* to swine. Swine are usually asymptomatic following Infection of swine with *T. gondii* are usually asymptomatic although abortions can occur if pregnant sows are infected. Diarrhea has also been produced in neonatal pigs (Lindsay et al. 1992).

Results from a serologic survey (Zimmerman et al. 1990) of swine farms in Iowa indicated a seroprevalence of 5.4% among finishing swine and 11.4% among breeding females. It was found that farms with less than 100 sows and gilts were more likely to be infected than farms with more than 100 sows and gilts. The in-herd seroprevalence of finishing swine tended to be lower if they were housed in confinement facilities. Seasonal variations in seroprevalence were not seen and seroprevalence tended to remain constant throughout out the year.

A state-by-state serologic survey to determine the prevalence of *T. gondii* antibodies has been reported (Kliebenstein et al. 1995). Three thousand four hundred and seventy-two serum samples were collected from 394 randomly selected swine farms. Overall sample prevalence of antibodies to *T. gondii* was 20%. The range of sample seroprevalence was 0% to 36%.

Although it is reasonable to believe that humans could become infected with *T. gondii* by ingesting pork containing oocysts, there is no epidemiologic research to support an association between pork and human toxoplasmosis (Zimmerman 1994).

Trichinella infections

Trichinosis is an infection caused by eating undercooked meat containing larvae of the parasitic worm, *Trichinella spiralis*. *T. spiralis* is considered to be less pathogenic for swine when compared to rats and humans (Corwin and Stewart 1992). Experimental infection of swine with *T. spiralis* results in decreased weight gain and apparent muscle soreness.

The overall prevalence of trichinosis in market hogs is very low at 0.1% (Bailey and Schantz 1986) and there appeared to be regional differences. There were no infections found in 3,245 hogs that were slaughtered in Minnesota, Wisconsin, Iowa, South Dakota, and North Dakota from 1983 to 1985. In contrast 0.7% of 5,315 hogs from New England states were infected.

Examination of all carcasses slaughtered in Denmark in 1994 indicated that all pigs were free from *Trichinella* infections. Swine from Denmark have been free of *Trichinella* since 1929 (Anonymous, 1994 #47). A serologic test for the detection of antibody produced in swine to *T. spiralis* has been developed for use in Denmark (Lind 1995).

Epidemiology of *Salmonella* infections in animals other than swine

Introduction

Ecology and environment play significant roles in the epidemiology of *Salmonella* infections and often affect the initiation, course, and outcome of the disease (Morse 1974). Increases in the reported cases of salmonellosis in food animals have been attributed to intensive food animal production systems (Glock and Schwartz 1993).

It has been shown that serotypes of *Salmonella* that are found in an animal product and associated with a human infection can also be found in the live animal source (Patterson 1972). Patterson (1972) reported the isolation of *Salmonella* 4,12:d:- from chicken carcasses and giblets. This serotype had also been isolated from poultry and humans in England. This finding indicated that there existed some common contacts among the human and poultry populations that allowed this serotype to be transmitted from farms to humans.

Environmental factors associated with *Salmonella* infections

The adaptive ability of *Salmonella* to various environments helps to understand the difficulty encountered with *Salmonella* control. *Salmonella* infections were reported to be more frequent in both human and lower animals during periods of high temperature and high relative humidity and that these factors favor the growth of *Salmonella* outside the host (Morse 1974). The optimal pH range for the growth of *Salmonella* has been reported to be 6.5 to 7.5. Weak organic acids and increased hydrogen ion activity appear to be deleterious to the growth of *Salmonella* (Morse 1974).

The survival of *Salmonella* in water, soil, manure and frozen poultry has been reported (Morse 1974). Aquatic environments are favorable for propagation of *Salmonella* (Morse 1974). For instance, *Salmonella paratyphi* antibodies have been found in fish. The following serotypes have been isolated from fish: *S. thompson, S. muenchen,* and *S. typhimurium, S. infantis, S. montevideo,* and *S. Amsterdam.* The survival of *Salmonella* in various water sources is variable. It has been reported that *Salmonella* survive for 87 days in tap water, 115 days in pond water, 120 days in pasture soil, 280 days in garden soil, over 30 months in dried bovine manure, 28 months in naturally infected avian feces, and 47 days in

manure slurry . Freezing reduces the total *Salmonella* numbers, but survivors may remain viable and infective for months. There are also reports of survival for up to 13 months in poultry carcasses that have been quick frozen at -37 °C and then stored at -21 °C.

Vectors as a source of *Salmonella* to animals

Salmonella have been reported to be carried in bats, sea gulls, flies, cockroaches, turtles (including pet turtles), dogs, and cats (Morse 1974).

Feed as a source of *Salmonella* to animals

A survey of animal by-products was conducted to determine the levels of *Salmonella* and other pathogens (Morehouse and Weman 1961). Seven hundred eighteen (718) of 5,712 feed samples collected from 31 states were positive for *Salmonella*. The isolations came from animal by-products and complete feeds. Fifty-one serotypes were reported. The four most frequently isolated serotypes were *S. montivideo, S. sentfenberg* (including diphasic*), S. typhimurium* (including *Copenhagen*) and *S. cubana*. Ingredient contamination ranged from 0% to 53% of the samples of each ingredient collected . Complete feed contamination ranged from 5% to 9.1%. Egg products, poultry by-products, meat scraps, meat scrap and bone meal and dog food accounted for 28% of the samples collected and 71% of the total *Salmonella* isolated. Two of four swine supplements were positive for *Salmonella*. It was believed that recontamination of product was the single most important factor accounting for the presence of *Salmonella* in animal by-products and that rodents were the most probable source of the recontaminating organisms. No conclusions were drawn about the association between disease and the presence of *Salmonella* in animal by-products. The significance of *Salmonella* in feed was unknown for the following reasons: 1. there was a general lack of

knowledge concerning the incidence of salmonellosis in the Untied States' domestic livestock population, 2. conclusive evidence was lacking that *Salmonella* serotypes responsible for disease in animals are introduced to livestock or poultry by their rations, 3. there was a wide variety of *Salmonella* serotypes isolated and the significance of this diversity is unknown, 4. attention should have been given to the numbers of organisms found in the by-products, 5. there were no standard methods of isolation used by laboratories, which made report interpretation difficult, 6. more work needed to be done to establish the pathogenicity of the various serotypes isolated in by-products.

A preliminary bacteriological survey of animal feeds and feed ingredients was conducted in the Winnipeg, Manitoba area (Isa et al. 1963) from which a total of 281 samples were collected and tested. There were 42 samples that were found to be contaminated with *Salmonella*. The following ingredients and the number of positive samples per total samples collected were tested as follows: meat meal (26/84), fish meal (1/11), soybean meal (4/42), bone meal (6/10), blood meal (2/13), crop meals (0/6), milk by-products (0/7), shorts, bran, and middlings (0/6), and urea (0/1). The following complete feeds and the number of positive samples per total samples collected were tested as follows: poultry feeds (2/47), pig feeds (1/33), dairy feeds, (0/17), and beef feeds (0/4). The four most frequently isolated serotypes were *S. worthington* (20), *S. newington* (8), *S. kentucky* (5), and *S. bredeney* (5).

The results of a survey of livestock and poultry feed was conducted to determine the levels of *Salmonella* contamination in four categories of feed ingredients (Allred et al. 1967). The four categories of feed ingredients and the number of positive samples per total samples collected were as follows: grains (18/2698), oilseed meals (60/2629), fish meal (38/805), and

animal by-product (270/869). The three categories of finished feeds and the number of positive samples per the total number of samples collected were as follows: cattle feed (22/2597), swine feed (49/1567), and poultry feed (84/1605). The five most frequently isolated serotypes and the number of isolations were *S. montivideo* (63), *S. eimsbuettel* (49), *S. senftenberg* (35), *S. cubana* (31), and *S. anatum* and *S. worthington* (27 each).

Feed ingredients and complete feeds from local feed manufacturers were examined over the course of two years (Patterson 1972). Sample prevalence differed among the plants that were sampled although no significance in the differences was noted. Samples of fishmeal (n=30), meat and bone meal (n=242), blood meal (n=36), poultry offal (n=100), and feather meal (n=5) had 1, 17, 2, 9, and 1 samples, respectively, positive for *Salmonella*. *Salmonella typhimurium* was the most commonly isolated serotype and all isolations were from meat and bone meal while *Salmonella* 4,12:d:- was the second-most frequently isolated serotype. Nineteen samples of cereals and vegetable protein meals contained no *Salmonella*. Nineteen samples of poultry feeds and 23 samples of cattle feed were negative for *Salmonella*. Three of 53 samples of pig feed were positive for *Salmonella*. The serotypes isolated from the pig feed were *S. typhimurium, S. infantis*, and *S. raus*. A dilution effect of compounding low inclusion rates of animal ingredients in the final product was attributed to the low prevalence. In this same study, *Salmonella* was also isolated from various stages of processing in one plant. It was suggested that processing temperatures may not always be high enough to reduce or destroy *Salmonella*. Leakage was noted from processing equipment that may have served as sources of contamination for subsequent batches of feedstuffs. The extent to which low level contamination in such feeds is responsible for *Salmonella* infection

in farm animals and poultry was not determined. It was suggested in this study that since *Salmonella* are likely to multiply if water is added to feed, low levels of feed contamination via the leakage of moisture from manufacturing equipment may be a risk for *Salmonella* infection in animals and poultry.

Between July 1990 and April 1991 the rate of *Salmonella* contamination in poultry feeds and feed components used by the Dutch feed industry was determined (Veldman et al. 1995). Samples of poultry feed (n=360) and poultry feed components (n=286) were collected and tested for *Salmonella* by culture. The total sample *Salmonella* prevalence was 12.7%; 16.8% of the feed component samples were positive for *Salmonella* while 9.4% of the complete feed samples were positive. Layer feeds, which were not pelleted, had the highest percent *Salmonella* positive samples (13.5%; 21 positive from 156 samples collected). Fish meal had the highest percentage of positive samples (30.8%; 40 positive from 130 samples collected) among the feed components. Meat and bone meal samples had a relatively low incidence of *Salmonella*. Pelleting of the poultry feeds significantly reduced the incidence of *Salmonella*. The most frequently isolated serotype from complete feeds was *S. agona* while the most frequently isolated serotype from the feed components was *S. hadar*. There was no association established among serotypes isolated in the feeds and those isolated in the feed components. However, it was still concluded that feeds constitute an important source of contamination of poultry.

Farm management practices associated with *Salmonella* infections

Factors that have been reported to contribute to *Salmonella* infections in animals include practices that would result in colostrum deprivation of neonates, overpopulation or

crowding, physical fatigue due to sorting, fighting, and movement, psychologic fatigue during the establishment of pecking orders, dietary changes, accidental introduction of poisons or toxins into a ration, loss of energy supply leading to building mechanical failure resulting in feed deprivation, lack of heat, light, ventilation, or humidity control, deworming or surgical manipulations such as castration or tail docking, accidental introduction of disease through breeding stock introductions, and transportation stresses (Glock and Schwartz 1993).

Risk factors

A review of environmental sources of *Salmonella* (Williams 1975) indicated that feed, fertilizers, water, humans, and wildlife can serve as sources of *Salmonella* for infection of livestock.

A multivariate analysis was used to determine a quantitative risk factor profile of salmonellosis for broiler breeder flocks (Henken et al. 1992). This study covered a five year period and involved 111 breeder flocks on 32 farms. A case was defined as a flock that had one positive isolation of *Salmonella* in a year. A control was defined as a flock that did not have any *Salmonella* isolations within the year. Hygiene, disinfection tubs, the interaction between hygiene and a disinfection tub and feed supplied from a small feed mill were the variables that were associated with the highest risk for the presence of *Salmonella*. Farms with poor hygiene barriers, no disinfection tub, and feed produced from a small feed mill had 46 times greater risk of being *Salmonella* positive. Flocks that used feed that had been manufactured in small feed mills meant flocks were 5.6 times more likely to be positive for *Salmonella*.

A chicken flock management survey was conducted among Canadian broiler flocks (Renwick et al. 1992) during 1989-1990. The prevalence of *Salmonella* isolations from floor litter or drinking water and the prevalence of floor litter samples that were positive for *Salmonella* were significantly associated with the age of the flock and the region of Canada in which the flock was located. *Salmonella* was most likely isolated from drinking troughs and plastic bell drinkers than from nipple drinkers. There were no significant associations with *Salmonella* isolation from floor litter or from drinking water when the following factors were examined: pest control, restrictions on visitors, clean-out methods, or water sanitizing.

Detection of *Salmonella*

An indirect ELISA was developed for the purpose of detecting antibodies to *Salmonella enteritidis* in chicken flocks (Nicholas and Cullen 1991). Two ELISA's were developed: one used lipopolysaccharide (LPS) extracted from *S. enteritidis* and a second used a heat extracted (H E) antigen from *S. enteritidis*. Both of these tests were compared for the detection of antibodies in groups of experimentally infected chickens and in chickens from 2 commercial flocks, one known to be infected with *S. enteritidis*. When the indirect ELISA using LPS was used to detect antibody individual samples, 237 of 239 known positive samples were detected to be positive. The HE ELISA detected all 239 known-positive samples as positive. Neither of the two ELISA's had positive reactions when tested with known-negative sera. Therefore, on an individual sample bases, the sensitivity and specificity for the LPS ELISA were .99 and 1, respectively. For the HE ELISA, sensitivity and specificity were both 1. The LPS and the HE ELISA tests were then used to detect antibody in 40 serum samples collected from birds from a flock that was known to be

positive for *S. enteritidis* and 40 birds from a flock known to have no history of *S. enteritidis*. The HE ELISA detected as positive all samples collected from the known-infected flock; the LPS ELISA detected only 25 of the 40 known-positive samples as positive for antibody to S. enteritidis. Neither of the ELISA's detected as positive any of the samples from the known negative flock. In the other flock of unknown status, none of the birds tested were culture positive for *S. enteritidis* nor were they seropositive as determined by either of the ELISA tests. When individual culture results were compared to the ELISA OD results from both tests using samples from culture-positive flock, there was no statistical correlation found between the presence of *S. enteritidis* as determined by culture and the OD results from either tests.

An ELISA was also developed for the detection of antibodies against *Salmonella* from serogroup D (Konrad et al. 1994). Cross-reaction with antibody against *Salmonellae* from serogroup B was noted. The reason for this cross-reactivity was postulated to be due to the presence of common lipopolysaccharide side chains of the O antigens of *Salmonellae* from both of these serogroups. Serogroup B is characterized by O antigens 1, 4, 5, and 12 while serogroup D contains O antigens 1, 9, and 12. To avoid or eliminate the cross-reactions, side chains 1 and 12 were cleaved from the group D LPS and used as the solid phase of the indirect ELISA. Cleavage of this side chain increased the specificity for detecting antibody against *S. dublin*. These results suggested that it could be useful for other *Salmonella* investigations and that cross reactions among serogroups do occur when using antibody assays such as an indirect ELISA.

Epidemiology of *Salmonella* infections in swine

General

Salmonella typhimurium (non-host adapted serotype) and *S. choleraesuis* (host-adapted serotype) are the two most frequently isolated from clinical cases of porcine salmonellosis (Wilcock and Schwartz 1992). Typically, septicemia results from infection by *S. choleraesuis* var. *kunzendorf*. A wide range of clinical signs are associated with infections by *S. choleraesuis* including fever, pneumonia, icterus, and toxemia with most consistent lesions being edema of lungs, splenomegaly, lymphadenopathy, icterus, and enterocolitis. Infections caused by *S. typhimurium* tend to be limited to the intestinal tract. Clinical signs associated with infection by *S. typhimurium* include an explosive diarrhea, dehydration and death due to necrotizing fibrinous enterocolitis (Reed et al. 1986); (Wilcock and Schwartz 1992).

Salmonella choleraesuis is an intracellular bacterium that tends to be transmitted by healthy infected carrier swine (Kramer 1995). Once ingested, *S. choleraesuis* attaches to and penetrates the intestinal mucosa and invades the lamina propria where it is phagocytized by macrophages. *Salmonella* choleraesuis is then spread throughout the body by these macrophages via the lymphatics: tonsils, Peyer's patches, gastric, hepatic, jejunal, ileocecal and bronchial lymph nodes. These organs serve are believed to as routes of entry as well as reservoirs for the persistence of *S. choleraesuis* in infected animals.

Morbidity and mortality in swine from *S. choleraesuis* infections are reported to be 50-80% and about 70%, respectively (Kramer 1995).

Salmonella typhimurium is shed by healthy carrier swine but may also be harbored by other animals and transmitted to pigs (Wilcock and Schwartz 1992). The severity of the enterocolitis produced by infection from *S. typhimurium* is variable (Wood et al. 1989). Once inside the pig, *S. typhimurium* pathogenesis is believed to be similar to that of *S. choleraesuis* mentioned previously. The presence of *S. typhimurium* in the various organs can then be shed into the environment.

Infection of swine with other serotypes of *Salmonella*

Swine have been reported to be naturally and experimentally infected with serotypes of *Salmonella* that have been associated with foodborne illnesses. *Salmonella agona* (Fernandez et al. 1993), *S. newport* (Wood et al. 1991), and *S. heidelberg* (Reed et al. 1985) have been reported to be capable of infecting pigs. Typically, when swine are infected with these serotypes, infection is characterized by diarrhea with or without severe clinical effects of the swine.

A recent report of the prevalence of *Salmonella* in finishing swine in the United States listed the three most frequently serotypes as *S. derby, S. agona*, and *S. typhimurium* which were isolated from fresh fecal samples from pen floors (Fedorka-Cray et al. 1996).

***Salmonella* in swine at slaughter**

In addition to the experimental and clinical studies regarding the infection of swine at slaughter with *Salmonella*, apparently healthy swine have been tested for the presence of *Salmonella* (Galton et al. 1954; Greenberg et al. 1963; Kampelmacher et al. 1963; Hansen et al. 1964; Lee et al. 1972; Hartwig and Jones 1976; Childers et al. 1977; Garcia et al. 1978; McKinley et al. 1980; Currier et al. 1986; Tay et al. 1989; Kramer et al. 1995; Fedorka-Cray

et al. 1996). Samples were collected from swine farms or from samples collected from swine carcasses at slaughter. Two of the more common serotypes isolated during these studies included *S. typhimurium*, and *S. choleraesuis*. However, other serotypes could be matched to the list of most frequently isolated serotypes from the Centers for Disease Control (Bean and Potter 1994) including *S. enteritidis*, *S. agona*, *S. heidelberg*, and *S. derby*. This suggests that there are serotypes of zoonotic importance present on farms in the United States and Mexico.

An outbreak of foodborne infection caused by S. infantis occurred in Copenhagen, Denmark area in 1994 (Nielsen et al. 1995). The sources of infection were pork products produced during a work stoppage at some slaughter facilities in Denmark. The demonstration of S. infantis in farms that supplied the slaughter facilities during this work stoppage provided an association between the presence of foodborne pathogens on farms the occurrence of foodborne outbreaks by the same serotype. A challenge facing epidemiologists and swine practitioners is developing a plan to reduce the prevalence of *Salmonella* on swine farms. Thus, it is important to have an understanding of the pathogenesis and monitoring methods used in studying *Salmonella* infections in order to make recommendations to swine farm managers that have a reasonable likelihood of reducing levels of *Salmonella*.

Pathogenesis of *Salmonella* infections in swine

***Salmonella choleraesuis* infection.** Infection of swine by *S. choleraesuis* is believed to begin via the ingestion of contaminated feces (Wilcock and Schwartz 1992). Experimental challenge studies have been conducted to determine the course of events following ingestion. *Salmonella choleraesuis* can be cultured from all parts of the digestive tract 16 h after infection with an oral dose of 1.2×10^{10} organisms (Reed et al. 1986). Isolation of *S.*

choleraesuis from the stomach was sporadic. Seventy-two hours after infection, the organism was found in the mesenteric lymph nodes. *Salmonella choleraesuis* was isolated from the lungs as 8 h after inoculation septicemia developed 48 h after inoculation. Infection of the spleen, kidney, and liver occurred 72 h after infection.

As part of this same study, 10^9 *S. choleraesuis* was inoculated into ligated intestinal loops. *S. choleraesuis* was isolated from mesenteric lymph nodes 2 hours after being inoculated into the ligated intestinal loops. These results suggest that the rate of spread of *S. choleraesuis* through the body of swine may be relatively rapid.

It has been demonstrated that there is a dose-related response following experimental infection of swine with *S. choleraesuis* (Gray et al. 1996). Three different challenge doses (10^3, 10^9, and 10^6) were given to susceptible pigs. Uninoculated swine served as negative controls. Rectal temperatures were taken from all pigs daily for 14 days after challenge and then weekly for the duration of the 15 week trial period. Individual swine from each treatment group were killed at 6, 10, or 15 weeks after challenge. Swine from the high-dose group were developed more severe clinical signs when compared to swine from medium-dose treatment group. Swine that were inoculated with 10^3 did not develop clinical signs associated with infection by *S. choleraesuis*. *Salmonella choleraesuis* was never isolated from tonsil, nasal, or rectal swab specimens or from pooled fecal samples from the low-dose group.

***Salmonella typhimurium* infection.** Infection by *S. typhimurium* is also believed to begin via ingestion of fecal-contaminated materials (Wilcock and Schwartz 1992). Oral inoculation of susceptible swine with 10^{10} *S. typhimurium* resulted in detectable organisms

throughout the digestive tract (except the jejunum) 8 h after inoculation (Reed et al. 1986). Mesenteric lymph nodes were positive 24 h after inoculation and septicemia was also reported 96 hours after inoculation (Reed et al. 1986). A second part of this same study included the inoculation of swine intestinal loops with *S. typhimurium*. Following the injection of 10^9 organisms into swine ligated intestinal loops, *S. typhimurium* was found in the mesenteric lymph nodes (2 h post-inoculation) and in the blood (8 h post-inoculation).

The influence of route of inoculation and dose on shedding and tissue distribution following infection of swine with *S. typhimurium* was reported (Fedorka-Cray and Stabel 1993). Pigs were inoculated with *S. typhimurium* either intranasally (IN) with 10^4 cfu/ml *S. typhimurium* (4-IN), or by gastric inoculation using gelatin capsules (GC) with either 10^4 (4-GC) or 10^6 (6-GC) cfu/ml. Tonsil, nasal, and rectal swabs were positive up to day 14 in the 4-IN pigs. Tonsil and rectal swabs collected from 4-GC pigs were positive 1 post challenge only; nasal swabs remained negative throughout the study. All pigs that were inoculated with 10^4 were tissue negative after 6 weeks. In the 6-GC group, tonsil and rectal swabs were positive for 12 weeks while nasal swabs were positive only through day 7. Tissues from the 10^6 group were positive after 12 weeks. These results suggested that dose and route may impact shedding of *S. typhimurium* since there were more organs that were culture positive in the 4-IN group than in the 4-GC group. It was also suggested that intranasal inoculation may result in widespread tissue distribution which may be attributed to both lymphatogenous and hematogenous routes of dissemination.

Alternate routes of invasion by *Salmonella* have been studied. In one study the role of the respiratory system was investigated (Fedorka-Cray et al. 1995). Pigs were

esophagotomized and challenged intranasally with *S. typhimurium*. After 3, 6, 12, or 18 hours following challenge, representative pigs were euthanized and necropsied. *Salmonella typhimurium* was reported from the following tissues 3 hours after challenge: turbinates, tonsil, mandibular lymph nodes, thymus, trachea, lung, bronchiolar lymph node, spleen, liver, middle ileum, cecum and colon. In addition to these tissues, *S. typhimurium* was isolated from cecal contents 6, 12, and 18 hours after challenge and from the ileocecal lymph nodes 6 and 18 hours after challenge. In another part to this study, pigs were anesthetized and infected by transthoracic injection of *S. typhimurium*. Three hours after the transthoracic injection, all tissues were positive by culture for *S. typhimurium*. These studies indicated that pigs can become infected with *S. typhimurium* via the respiratory route of infection in addition to fecal/oral infection.

Duration of shedding

The length of time after inoculation that *Salmonella* is shed in feces is variable. In a study of the effect of antibiotic feeding to swine infected with *S. typhimurium* (Wilcock and Olander 1978), *S. typhimurium* was isolated from composite pen feces and fecal swabs for up to 120 days after infection.

The duration of persistent infection by *S. typhimurium* has been reported (Wood and Rose 1992). *Salmonella*-free pigs were orally exposed to *S. typhimurium* and necropsied at various intervals during a 28 week study period (Wood and Rose 1992). It was shown that pigs that were necropsied at 28 weeks of age were culture-positive for *S. typhimurium*. Although the 28-week old pigs were clinically normal, low (10 - 100 organisms per gram of

tissue) numbers of *S. typhimurium* were detected in the cecal wall, cecal contents, ileum, ileocecal lymph nodes, colon wall, colon contents, tonsils and mandibular lymph nodes.

The duration of shedding of *S. typhimurium* following experimental challenge has also been reported (Wood et al. 1989). Seven pigs were cultured for the presence of *S. typhimurium* 28 weeks post challenge. *Salmonella typhimurium* was cultured from fecal, tonsil swab, and rectal swab samples from 1, 5, 1 pigs, respectively, 24 weeks after challenge.

The duration of shedding of *S. choleraesuis* following experimental challenge has been reported (Gray et al. 1996). No *S. choleraesuis* were cultured from samples collected from pigs that were challenged with 10^3 nor negative, unchallenged control pigs. Swine that received 10^9 or 10^6 shed *S. choleraesuis* for 15 or 9 weeks (respectively) as determined by culture individual rectal swabs and pooled pen feces.

Detection of *Salmonella* infections in pigs

Regular pen fecal samplings were collected from occupied pens in buildings on a swine farm that was experiencing an epizootic of salmonellosis (Heard and Linton 1966). A comparison of rectal swabs and pen fecal samples for the purpose of determining the pen or building status indicated that neither method was superior for this purpose. Seventeen pen fecal samples gave similar pen prevalence results as did the collection and culture of 104 rectal swabs. Therefore, in order to reduce the number of samples needed for collection, only pen fecal samples were collected for the duration of the study.

Bacteriologic culture of pigs has been the preferred method used determining whether or not groups of pigs have been infected with *Salmonella* Various culture methods were

compared for their ability to isolate *Salmonella* from pigs (Bager and Petersen 1991). Three different selective enrichment media were compared: Rappaport-Vassiliadis broth (RV), selenite broth (SB) and Müller-Kauffmann tetrathionate broth (MKTB). The culture material used for this comparison was swine feces. Fecal samples were pre-enriched in buffered peptone water and then aliquots were transferred to RV, SB, and to MKTB. After incubation, aliquots from all of these broths were inoculated onto modified brilliant green agar (BGA). The evaluation of each medium was made by comparing results to the true status of the sample. The true status of the sample was defined as whether or not *Salmonella* was detected by any method of enrichment. The sensitivity of RV was higher than SB or MKTB at either incubation time of 24 or 48 h. Also, it was suggested that MKTB was somewhat toxic for *S. typhimurium*.

Three different protocols were compared for the culture of *Salmonella* from swine feces (Fedorka-Cray et al. 1995). Fresh fecal samples were collected from the pens of finisher age pigs. In one protocol (T48-R) approximately 1 g of feces was inoculated into TET and then incubated for 48 h at 37 °C. Then, 100 µl were transferred into Rappaport R-10 (R-10) and incubate at 37 °C for 24 h. Following incubation, R-10 cultures were struck onto xylose-lysine-tergitol 4 (XLT-4) agar, brilliant green agar with sulfadiazine (BGS) and brilliant green agar with novobiocin (BGN) agar and incubated at 37 °C for 24 h. In the second protocol (T-48) TET cultures were not inoculated into R-10 but also struck onto XLT-4, BGS, and BGN and incubated at 37 °C for 24 h. In the third protocol (GN-R), feces were inoculated into GN-Hajna broth and incubated at 37 °C for 24 h. Then, 100 µl were inoculated into R-10 and incubated at 37 °C for 24 h. Then, inoculum from R-10 was struck

onto XLT-4, BGS, and BGN agar. Colonies typical of *Salmonella* were picked and inoculated into triple sugar iron and lysine iron agar slants. *Salmonella* were most often isolated from samples that were cultured according to the T48-R protocol. There were no differences noted among the plating media.

Phage typing and plasmid profiling have been evaluated for use as epidemiological tools in the investigation of *S. typhimurium* infections in swine (Bager and Baggesen 1992). The most common *Salmonella* serotype isolated from Danish swine is *S. typhimurium*. Phage type 12 was isolated most frequently from isolates of *S. typhimurium* from clinical isolations as well as incidental isolation. Plasmids coding for virulence were found in isolates originating from clinical and nonclinical herds. These findings led the investigators to conclude that clinical outbreaks of porcine salmonellosis reflect the presence of precipitating causes rather than the presence of the organisms. Within a herd, there was very little variation of the *S. typhimurium* isolates with respect to phage type and plasmid profile. Phage type and plasmid profile seemed to remain quite constant over time within a herd. Also, because phage type 12 and plasmids of 90 and 4.3 kd. predominated in the *S. typhimurium* isolates from many different herds, it was suggested that these markers were of limited use for epidemiological investigations of *S. typhimurium* in Danish pig herds.

An indirect ELISA was developed for use as a screening test for groups of pigs infected with *Salmonella* (Nielsen et al. 1994; Nielsen et al. 1995). The test was referred to as mix-ELISA because the antigen used on the solid phase was lipopolysaccharide (LPS) extracted from *S. typhimurium* (O:1,4,5,12) and *S. choleraesuis* (O:6,7). Lipopolysaccharide from *S. choleraesuis* was used because it bound with *S. typhimurium* LPS to microtiter plates

better than the LPS from *S. infantis*. Pigs were infected with *S. typhimurium* and bled several times over the course of 110 day study period. The serologic response to *S. typhimurium* was detected 7 days post challenge, peaked at about 30 days post challenge and gradually declined for the duration of the study. Fecal samples collected from the pigs during the study were cultured. The percent of fecal shedders was highest on the first day after challenge and gradually declined to zero until day 94 of the study when there was a slight increase in the number of fecal shedders. Fecal shedding returned to zero percent shedding by the end of the study. Results from the mix-ELISA were reported as OD% which was calculated as follows:

OD% = [(Calibrated Sample OD - 0.176)/3.068-0.176) X 100]

Reference sera were produced from non-infected pigs (negative reference sera) and pigs infected with *S. typhimurium* or with *S. infantis* (positive reference sera). The reference sera were then used to determine the background OD's . Serum from negative had an OD of 0.176 and a known positive reference serum had an OD of 3.068. These values were determined after analysis of sera from a negative pig (0.176), 4 sera from *S. typhimurium*-infected pigs (0.854, 1.337,1.758, 1.789), and 2 sera from *S. infantis*-infected pigs (2.167, 3.068). The negative serum sample was used as the negative reference sample and the serum sample with the highest OD (3.068) was used for the positive reference sample. Thus, these values were used as the constants in the calculation of OD% in the formula above.

The premise for the use of LPS from *S. typhimurium* and *S. choleraesuis* is that the O-antigens from *S. typhimurium* (1, 4, 5, 12) and *S. choleraesuis* (6, 7) are contained by 90% of the serotypes of *Salmonella* that have been associated with human foodborne illnesses in Denmark (Nielsen et al. 1995). Attempts were made to use LPS from S. infantis in the mix-

ELISA but it failed to adhere to the microwell plates when mixed with the LPS of *S. typhimurium*. Therefore, another serotype with the same O-antigen content (*S. choleraesuis*) was used because it's LPS did adhere to the microwell plates when mixed with the LPS of *S. typhimurium* (Nielsen et al. 1995). The specificity and sensitivity of the mix-ELISA are .9 and .96, respectively (Nielsen, personal communication).

The mix-ELISA was then used in a surveillance study to determine its usefulness in detecting herds with high levels of *Salmonella* in Denmark (Nielsen et al. 1995); (Nielsen et al. 1996). Sera from 10 *Salmonella* culture negative swine herds in Denmark and 28 *Salmonella* culture positive herds (27 Danish herds and 1 herd from the United States) were examined. Serotypes isolated from the culture positive herds included *S. bredeny, S. choleraesuis* (from the United States herd), *S. derby, S. enteritidis, S. infantis, S. livingstone, S. panama, and S. typhimurium*. Some of the 10 culture-negative herds had seropositive individuals but the number of seropositive individuals per farm never exceeded 21%. The mean OD% readings for each of the culture-negative farms was low (OD% = 3). In contrast, herds infected with *Salmonella* had 22-87% seropositive individuals with herd mean OD% ranging from 9-48%. This study indicated that a wide variety of *Salmonella* serotypes will produce an antibody response that is detected by the mix-ELISA. This study did not report the correlation between individual culture and serum sample results. The only parameters measured were the overall farm culture status and the overall farm serologic status

The presence of antibody to *Salmonella* in meat juice was compared with antibody present in serum of pigs at slaughter (Nielsen et al. 1996). When OD% cut-offs of 10, 20, and 30 were examined, meat juice/serum ratios between 0.89 and 0.95 were calculated. The

specificity and sensitivity of meat juice using serum ELISA as a reference resulted in a specificity between 0.95 and 0.99 and a sensitivity between 0.81 and 0.89. This study indicated that meat juice could be used to monitor the *Salmonella* status of swine herds.

Salmonella in feedstuffs and feeds used in swine

Many reports have been written in which the presence of *Salmonella* was demonstrated in feed and feedstuffs used for swine. A review of two papers associated with feed isolation of *Salmonella* has been published (Williams 1975); (Malmqvist et al. 1995). *Salmonella* isolated from feeds and feedstuffs can be isolated from pigs fed diets containing *Salmonella*-contaminated ingredients.

Angola fish meal and Pakistan bone meal that had been naturally contaminated with *Salmonellae* were used to formulate diets to be fed to *Salmonella*-free pigs (Smith 1960). Control pigs were housed separately and fed the same diet without the fish meal and the bone meal. Eighteen different serotypes of *Salmonella* were found in the contaminated meals. Rectal swabs were collected periodically from each pig and cultured for *Salmonella*. At regular intervals, pigs from the experimental group were euthanized and necropsied and organ samples were collected at necropsy and cultured for *Salmonella*. Surviving pigs from the experimental group were changed to the control diet 50 days after being fed the contaminated diet. Twenty days later, these pigs were euthanized and necropsied. Four of the five serotypes isolated from mesenteric lymph nodes were also found in the meals. Ten of the 12 serotypes found in the rectal swabs were also found in the meals. This study demonstrated that contaminated feed can be a source of *Salmonella* infection to pigs. These

findings also implied that there is a risk of meat becoming contaminated by alimentary contents at slaughter which could result in exposure of humans to *Salmonella*.

The prevalence of *Salmonella* in swine feed in the United States has recently been reported (Harris et al. 1997). Thirty swine farms from 8 states were sampled by the veterinarian who routinely visited the farm for swine health purposes. In addition to samples of complete feed and ingredients, a herd questionnaire was developed by the investigators and completed by the herds' attending veterinarians. Complete feeds and ingredients were sampled and cultured for the presence of *Salmonella*. The sample prevalence of *Salmonella* was 2.8% while the farm prevalence of *Salmonella* in the feed and ingredients was 46.6% (14 of 30). Thirteen different serotypes were isolated. The most frequently isolated serotype was *S. worthington*. The presence of *Salmonella* in feed and feed ingredients was significantly associated with no bird-proofing, the on-farm (as opposed to purchased feed) production of feed for finishing pigs, and failure to use confinement facilities in grower, finisher, gestation, and breeding sections of the herd.

Survival of *Salmonella* associated with infection in swine

The survival *of S. typhimurium, S. choleraesuis, S. choleraesuis* var. *kunzendorf,* and *S. typhisuis* in various environmental conditions have been studied (Morse et al. 1982). These serotypes were inoculated into pasture sod from which samples were collected at various intervals for the duration of a 520 day period. The first three serotypes persisted for 451 days *while S. typhisuis* persisted for 35 days. In addition, *S. typhimurium* survived in dried feces from experimentally infected swine for 291 days. All four serotypes persisted for 7 to 14 days in aerobic and anaerobic soil and water mixtures. *Salmonella typhimurium* was

isolated for 56 days following inoculation into anaerobic sediment from soil and water mixtures. In antimicrobial-free swine manure and water mixture, S. *typhisuis* survived for 7 days in aerobic conditions and 28 days anaerobically. *Salmonella choleraesuis* and *S. choleraesuis* var. *kunzendorf* survived for 56 days in the sediment of an anaerobic manure system. *S. typhimurium* was isolated after 173 days in the bottom sediment of an anaerobic manure system. These results demonstrate the ability of at least some serotypes of *Salmonella* to persist in the environment. Thus, environmental sources of *Salmonella* must be considered when conducting epidemiologic studies of *Salmonella* and instituting *Salmonella* control programs.

Risk factors associated with *Salmonella* infections in swine

Culture of pen feces was used to monitor the progress made in an attempt to reduce the amount of *Salmonella* in a closed pig herd (Heard and Linton 1966). The purpose of the study was to determine if pigs that had been declared free of *Salmonella* by culture could be transferred to sanitized pens that had been occupied by *Salmonella*-positive pigs and remain free of *Salmonella*. Careful attention to sanitation, pig movement, and people traffic were monitored in this study. Following transfer, pigs in only 6 pens became reinfected after placement. The sources of infection were associated with direct contact with feces of infected pigs following movement, mixing of pens, or by service personnel entering the pens. It was concluded that *Salmonella* infections could be reduced by careful attention to sanitation and pig movements.

The following factors were reported to be important considerations in the control of *Salmonella* infections in a closed swine herd (Heard and Linton 1966; Heard et al. 1968):

use of appropriate medications in the face of an outbreak, restriction of pig movement, disinfection, heat treatment of feed, vaccination, structural changes within a building to reduce exposure of neighboring pigs, and elimination of the practice of mixing pigs. Again, it must be emphasized that certain farm management practices can be successfully applied to swine herds for the purpose of controlling *Salmonella* infections.

A computer-based analysis of epidemiological data collected from a herd that had experienced an outbreak of salmonellosis has been reported (Heard et al. 1968). The main route of cross-infection was contact with other infected pigs. Infection tended to occur more frequently when infected and non-infected pigs were mixed in pens and at all times of service. Uninfected animals placed in disinfected pens and not moved from those pens tended to remain free of *Salmonella* infection. This finding suggested that little cross-infection occurred between pens. It was also reported that of all pigs found to be excreting *Salmonella* on one or more occasions, over 50% of those same animals ceased shedding *Salmonella*. Two factors that corresponded with a high incidence of infection were the number of pigs per pen and the age group of pigs. Pens with more than 7 pigs and pigs in the 3 to 7 month old age group tended to have higher incidence of infection. Concurrent disease and associated chemotherapy, vaccination and the use of a common weighing pen had little or no influence on the incidence of infection.

Outbreaks of salmonellosis in 7 finishing herds were traced back to the sow herd source (Bager and Baggesen 1992). In one instance, one sow herd and all three finishing sites it supplied were shown to be positive for *S. typhimurium*. A similar finding was made with another sow herd supplying a finishing unit that had experienced an outbreak of *S.*

typhimurium. However, in 6 finishers where *S. typhimurium* infection had occurred, none of the sow herds supplying the finishers was positive for *S. typhimurium*. One sow farm was shown to be positive for *S. typhimurium* as well as a finisher building on the same farm. However, an off-site finisher supplied by the same sow herd was not shown to have *S. typhimurium*. Negative results could have been due to the poor sensitivity of conventional bacteriological methods used. Conversely, the negative results may suggest that vertical transmission of *S. typhimurium* can be broken in some herds.

Mice have been implicated in maintaining the persistence of *Salmonella* on pig farms (Duhamel et al. 1993). Seven Midwest swine farms with a history of salmonellosis were chosen for the study. At each farm visit, mice were collected that had been captured with multiple catch traps and at least two chronically ill pigs were euthanized and necropsied. *S. choleraesuis, S. typhimurium, S. agona,* and *S. anatum* were isolated from pigs, mice, and environmental samples from 5 farms. *Salmonella* were not isolated from two of the farms. On one farm, *S. choleraesuis* was isolated from pigs and mice while *S. typhimurium* was isolated from pigs only. On a second farm, *S. choleraesuis* was isolated from pigs only while *S. typhimurium* was isolated from mice, pigs, and environmental samples. On a third farm, *S. agona* was isolated from pigs, the environment and mice. On the remainder of the farms, *S. typhimurium* was isolated from pigs only and *S. anatum* was isolated from environmental samples only. On two farms with a history of *Salmonella* infections, no *Salmonella* were isolated from captured mice. Other risk factors that have been identified as important in the epidemiology of porcine salmonellosis include increased population densities, reduced sanitation, and an increase of other stressors (Glock and Schwartz 1993).

Flooring type was compared in a cross-sectional study of *Salmonella* prevalence in a swine farm (Davies et al. 1997). Pigs from one farm were finished in one of two types of buildings, characterized by the flooring: open-flush gutters or slotted flooring. It was found that pigs housed in the building with an open-gutter flush had higher culture and serologic prevalence of *Salmonella*. This study also suggested that groups of pigs having a high culture prevalence of *Salmonella* also have a high serologic prevalence of *Salmonella* antibody. Thus, the use of serologic monitoring could be useful to determine the prevalence of *Salmonella*.

Control of *Salmonella* infections

Farm management practices associated with control of *Salmonella* infections in swine

Successful control and elimination of salmonellosis a closed swine herd has been reported (Heard et al. 1968). After an outbreak of clinical salmonellosis caused by *S. typhimurium*, attempts were made to eliminate the infection from the farm over a three-year period. There were three periods of the program. The first began with the treatment of the initial outbreak with medications in the feed of all pigs and the treatment of all clinical cases. At the same time that medications began, pig movements to exercise pens and to a communal scale were banned. Communal scales were used only when it was possible to weigh animals from one section of production and after scales had been cleaned and disinfected. During this initial outbreak, all pig feed was heated to at least 145° F for 10 minutes and a formalin-inactivated bacterin containing *S. typhimurium* was given for one year to all pigs at the age of 4 weeks with a booster given at 6 weeks of age. Finally, the communal dunging area of each building was changed so that no pen had immediate contact with feces from adjacent pens.

In addition, solid pen partitions of rendered block were built to 3 feet 6 inches between pens. During the second phase of the control program, in addition to implementation of period one changes, pen fecal samples were collected every two weeks for 16 weeks and repeated 5 months later for a period of one year. At the beginning of the third period, all bacteriological results revealed the major pathways of cross infection. Based on these findings, further restrictions of the movement and mixing of pigs were imposed. The results of these measures brought about a reduction in the number of *Salmonella*-positive animals that were sent to slaughter. The isolations of *Salmonella* fell from 8.22-12.3% shortly after the management changes were implemented to 0% for 6 consecutive samplings over a period of 6 months.

Central to any *Salmonella* control for swine are management practices that should probably be in place on all swine farms. In addition, methods for monitoring the success associated with these management practices should also be part of the day-to-day operations of a swine farm. The following management practices have been considered important in the control of salmonellosis in swine (Fedorka-Cray 1993): rodent control, maintained ventilation systems, cleaning and disinfection, restriction of the movement of pigs, footbaths, boots and coverall changes between groups of pigs, personal hygiene, all-in all-out pig flow, and possibly vaccine use. Monitoring methods suggested in this review included culture of the environment and culture of animals followed by removal of positive animals to separate facilities where treatment may be instituted. It has been suggested that rodent control and disinfection along with all-in all-out management systems are essential for effective control of salmonellosis in swine (Duhamel et al. 1993).

When confronted with a swine herd that is positive for *Salmonella*, one must be concerned with whether or not management changes can be instituted to reduce the levels of *Salmonella* on the farm. Controlling *Salmonella* infections on farms would require knowledge of the factors that can contribute to its spread with in a herd. The distribution of *Salmonella* in herds with different levels of clinical disease has been studied (Baggesen et al. 1996). Twelve herds were studied: 3 with clinical disease, 6 with subclinical disease, and 3 without known *Salmonella* problems served as controls. Samples of various environmental material were collected from each section of the herd and cultured for the presence of *Salmonella*. Sections of herds sampled included the sow barns, farrowing, nursery, grower and finishing buildings. Material sampled included pig feces, pen feces, dust, swabs from equipment and ventilation, and slurry. *Salmonella* was isolated from all material with the most frequent isolations coming from slurry (34%), pig feces (25%) and pen feces (24%). *Salmonella* was isolated from known positive herds and two control herds. Two serotypes of *Salmonella* were isolated from two known positive herds. Pen samples proved to be useful for monitoring the bacteriological status of the pigs. Herds with clinical disease were highly contaminated with *Salmonella* throughout all phases of production. However, herds that had no clinical problem associated with *Salmonella* infection had a lower frequency of isolation. Environmental sanitation was considered by the investigators to be a critical control point for the reduction of *Salmonella* in swine.

The spread of *Salmonella* infection in pigs is thought to occur from contact of pens housing infected pigs with uninfected pigs in adjacent pens. The possibility of raising uninfected pigs in the same facility that housed *Salmonella*-infected pigs was investigated

(Dahl et al. 1996). Three *S. typhimurium*-infected pigs from a herd which had been shown to be positive for *S. typhimurium* were transferred to an isolation facility and housed in one pen. Eighteen pigs were also taken from a *Salmonella*-negative herd and placed in 3 pens. Seven pigs were placed into each pen adjacent to the infected pigs, and 4 pigs were place in the same pen as the 3 infected pigs (n=7). Solid partitions were placed between the adjacent pens. At the end of a 73-day trial period, pigs were necropsied. Only one pig in one the adjacent pens seroconverted to *Salmonella*. All fecal samples from the same pigs and from the pigs in the distant pen were culture-negative for *Salmonella*. Physical barriers between infected and noninfected pigs appeared to reduce the spread of *Salmonella* to uninfected pigs.

Eradication of *S. typhimurium* has been attempted (Dahl et al. 1996). The purpose of this study was to determine if the removal of pigs from a *Salmonella*-infected source farm could eliminate *S. typhimurium*. Three herds with persistent *S. typhimurium* infection were selected for this study. Pigs from Farm A were weaned at 21 days of age and moved to an off-site facility for growth to slaughter. On the other two farms, pigs were removed at 10 weeks of age (Farm B) or pigs were removed directly from the grower building to separate facilities for growth to slaughter (Farm C). All pigs from Farm A that were weaned at 21 days of age and moved off-site remained seronegative until slaughter. In contrast, all but one of the control pigs that remained on the source farm (A) seroconverted. No *S. typhimurium* were isolated from the off-site pigs from Farm A but by 16 weeks of age, *S. typhimurium* was isolated from all samples of the control pigs that remained in the finisher of Farm A. Pigs that were removed from Farm B at 10 weeks of age remained bacteriologically and serologically negative while 10 of 88 control pigs that remained on the source farm were serologically

positive. At slaughter, *S. typhimurium* was isolated from the cecal contents of one of the control pigs from Farm B at the time of slaughter. All pigs removed from Farm C remained serologically and bacteriologically negative. The nursery and grower sections of Farm C remained culture-negative for *Salmonella* after being cleaned and disinfected between groups of pigs. The finishing facility of Farm C remained culture positive for *Salmonella* and 16 of 30 control pigs left on the source farm seroconverted. This study demonstrated that it was possible to procure pigs from infected herds and rear them free of *S. typhimurium*. It was suggested by the investigators that it was important to clean and sanitize between groups of pigs and that a change of pig flow from continuous to all-in all-out were beneficial in eliminating *S. typhimurium*.

The inclusion of acidic byproducts of the dairy industry (whey) in the feed of swine has been associated with reduction of fecal shedding of *Salmonella* in swine (vanSchie and Overgoor 1987). Forty swine farms were studied. Twenty farms fed pellets or crumbles with water added for moisture while in another 20 farms whey was used as a source of moisture for swine feed. Over half (56.2%) of the fecal samples from the farms that used water were positive for *Salmonella* while 10.2% of the samples from farms that used whey to moisturize swine feed were positive for *Salmonella*. Most (80%) of the farms that used water were positive for *Salmonella* while 40% of the farms using whey were positive for *Salmonella*. These differences were significant and demonstrated the association between feeding acidified dairy products and reduced fecal shedding of *Salmonella*.

The effect of organic acid treatment of feed on seroprevalence and shedding of *Salmonella* in finishing pigs was investigated (Dahl et al. 1996). A mixture of formic,

propionic, ammonium formate and ammonium proprionate was fed at the rate of 4000 ppm to pigs on a farm with a history of *S. typhimurium*. Control pigs on the same farm were fed the same diet without the organic acids. There were no measurable difference in shedding or seroprevalence when the two treatment groups were compared suggesting that there is no benefit to using this acid combination for controlling *Salmonella* infections.

A model for *Salmonella* reduction swine herds was developed and tested in Denmark (Dahl et al. 1996). The model consisted of microbiological surveying of a herd to identify infected areas within the herd with subsequent development of a plan to reduce *Salmonella*. The plan consisted of improved hygienic measures and a change from continuous pig flow to all-in all-out pig flow. Pig flow changes were attempted at either the pen level or the building section level. On some occasions, organic acids were used as an intervention step. Thirteen studies were conducted in 11 herds. In one herd, a low prevalence of *Salmonella* was achieved before interventions could be instituted. This suggested that some infections might resolve spontaneously or that other factors associated with infection were not identified. In the other 10 herds, 7 successfully reduced the seroprevalence of *Salmonella* by various combinations of pig flow changes and the inclusion of organic acids in the feed. In one herd, three attempts were made to reduce the prevalence of *Salmonella*. The first two attempts to improve pig flow were unsuccessful in reducing the prevalence of *Salmonella*. However, in the third attempt to intervene, organic acids were used in addition to the pig flow changes to successfully reduce the levels of *Salmonella*. No explanation was offered for the failure to reduce the levels of *Salmonella* in the other two herds. The investigators concluded that there may be some herds in which the reduction of *Salmonella* may not be possible.

Not all attempts to reduce the levels of *Salmonella* in pig farms are successful. Failure to accomplish a reduction in levels of *Salmonella* has been attributed to failure to completely clean facilities in some cases. In situations where reduction appears to be quite difficult, it has been recommended that a farm consider changing production facilities so that different sites are used to raise pigs (Dahl et al. 1996); (Dahl et al. 1996).

The role of medication of replacement animals for the purpose of eliminating *S. typhimurium* was studied (Dahl et al. 1996). This study evaluated the benefits of medicating gilts prior to introduction into a breeding herd. Gilts (n=30) from a herd with a history of *Salmonella* infection were treated with enrofloxacin for four days prior to removal to cleaned and disinfected facilities. Blood samples from all gilts were collected 3 and 44 days after transport. Fecal samples were collected from 17 gilts 57 days after transport. *Salmonella typhimurium* was isolated from the fecal samples of 2 of the 17 gilts. Seroconversion measured by the Danish mix-ELISA occurred in 26 gilts on day 4 and 27 gilts on day 44 after transport. This study suggested that medication of pigs before and after introduction into a herd will not reduce the seroprevalence of *Salmonella* or eliminate *Salmonella* from gilts.

Control of *Salmonella* in feed and feedstuffs used in swine

Reports from as early as 1967 suggested that a reduction in *Salmonella* contamination of animal byproducts would lower the *Salmonella* contamination in swine and poultry feeds (Allred et al. 1967).

Sweden has a national control program designed to prevent *Salmonella* from contaminating food (Malmqvist et al. 1995). Following are some of the salient features of the Swedish *Salmonella* control program. This program has resulted in the rare occurrence of

salmonellosis in humans and low occurrences of salmonellosis in domestic animals. Central to this control program is the bacteriologic testing of ingredients used in the formulation of diets for consumption by domestic animals. All isolations of *Salmonella* must be identified and reported to the Swedish Board of Agriculture (SBA). Official monthly summaries of each report are published by the SBA. *Salmonella* infections in swine have decreased since the reporting period of 1978-1982. Testing of feed in Swedish feed mills involved end-point testing of complete feeds by collecting one sample for each 100 tons manufactured. In 1991, end-point testing was replaced with the Hazard Analysis Critical Control Point system. This system increased the number of samples collected and, therefore, the number of *Salmonella* isolations increased. Five or two samples of complete feed are collected weekly from poultry, cattle, and swine feed mills. When positive samples are detected, further testing in the mill is conducted and necessary sanitation procedures are implemented. Most positive samples are collected from the unloading areas and the intake elevators of the feed mills. Very few positive samples are collected from inside the pellet cooler or from the top of the bins used for storage of ready-mixed feeds.

Role of antibiotics in the course of porcine salmonellosis

Ampicillin was used as a therapeutic by one veterinarian in an effort to eliminate *Salmonella* from a closed herd (Heard et al. 1968). However, in a side-by-side study within the control program, it was found that the oral or parenteral injection of ampicillin to known infected pigs failed to produce a significant reduction in excretion rates of *S. typhimurium* (Heard et al. 1968). It was suggested that the failure of ampicillin to reduce shedding may have been due to continuous exposure of the pigs to a contaminated environment, resulting in

reinfection of the pigs. However, such conditions of continual exposure are typical on swine farms and suggest the dubious role of antimicrobials for the purpose of reducing the number of swine that shed *Salmonella*.

The influence of oral antibiotic feeding on the duration and severity of clinical disease, growth performance and the pattern of shedding in swine inoculated with *S. typhimurium* was examined (Wilcock and Olander 1978). Various regimens of feed and water medication were administered to pigs at the time of challenge or during the period of diarrhea. None of the methods of antibiotic administration affected the duration or recurrence of diarrhea in pigs inoculated with *S. typhimurium*. Also, antibiotic treatment for enterocolitis caused by *S. typhimurium* did not show any clinical benefit or reduction in the frequency or duration of fecal shedding by pigs that recovered from clinical illness. It was concluded that feed antibiotics probably do nothing to reduce the hazard posed by the carrier pig to animal caretakers, other livestock, or consumers.

The antimicrobial sensitivity pattern of *Salmonella* isolates from swine in Kansas indicated that an increasing number of *Salmonella* isolates were becoming resistant to carbadox, a medication frequently used to control salmonellosis in swine in the United States (Mills and Kelly 1986). Serotypes of *Salmonella* that were tested included *S. choleraesuis, S. typhimurium (*including *copenhagen), S. derby, and S. anatum*. Antibiotic effectiveness *in vitro* was compared as follows: greater than 90% sensitive for gentamicin, sulfamethoxazole with trimethoprim, sulfachoropyridazine, nitrofurazone; greater than 80% but less than 90%, chloramphenicol, furazolidone, neomycin, and kanamycin; tetracycline (59% sensitive), carbadox (50% sensitive), and triple sulfonamides (sulfadiazine, sulfamethazine, and

sulfamerazine, 17% sensitive). These results suggested that empirical use of antimicrobials in the face of an outbreak of salmonellosis in swine may result in less than adequate treatment of affected individuals.

Vaccines used to control *Salmonella* infections in swine

As part of an epidemiologic analysis of an outbreak of *S. typhimurium* in a closed herd of pigs, the use of vaccine was evaluated as a tool to reduce the shedding *of S. typhimurium* (Linton and Heard 1970). A killed *S. typhimurium* vaccine had little effect on the incidence of *Salmonella* excretors.

An attenuated strain of *S. choleraesuis* has been reported to be beneficial as a vaccine to protect pigs against the development of clinical disease from infection by virulent *S. choleraesuis* (Kramer et al. 1992). This strain of *S. choleraesuis* (Scs 54) was found to be safe when inoculated into pigs. There were no deaths reported when the vaccine was administered by gastric lavage compared to three pigs that died after being challenge with the parent strain of *S. choleraesuis* (Scs 38). Only 7/50 organ suspensions from the vaccinates were positive for *Salmonella* while 33/49 organ suspensions were positive for *Salmonella* in the Scs 38 group. Additionally, the pigs from a herd with active paratyphoid that had been vaccinated with Scs 54 gained more weight (100 grams during 18 days) and had fewer deaths (0) when compared to controls from the same herd which received either an autoclaved preparation of the Scs 54 vaccine or a starch suspension in lieu of vaccine strain Scs 54. In a second efficacy study, vaccinates also had fewer deaths (0), increased weight gain (344 grams per 14 day observation period) when compared to the nonvaccinated pigs (3 deaths, 95 grams average weight loss during the 14 day observation period). These differences, as

well as differences in rectal temperature during the 14 day observation period, were significant. The vaccine strain Scs 54 was administered to pigs in various field trials. Administration of the vaccine was via drinking water in the face of a clinical outbreak of paratyphoid. Although there were deaths immediately preceding vaccination and 2 days following vaccination, there were no deaths reported from day 4 after vaccination in the drinking water. These results showed that vaccine strain Scs 54 was effective in protecting pigs against challenge by virulent *S. choleraesuis*. In addition, there was no evidence that Scs 54 reverted to virulence nor adversely affected vaccinated pigs.

The immune response of pigs that had been vaccinated with an aromatic-dependent mutant of *S. typhimurium* was studied (Lumsden and Wilkie 1992). Within a litter, pigs were randomly assigned to one of two treatment groups: vaccinated or unvaccinated controls. Treatment and litter effects were observed when lymphocyte blastogenesis response was measured to O-polysaccharide (O-ps), whole bacterial cell agglutination, O-ps hemagglutination, and LPS hemagglutination and compared among vaccinates and nonvaccinates. Vaccination significantly affected the lymphocyte blastogenesis response but not the serum antibody response following challenge. There was a significant litter effect when responses to the primary and secondary post-vaccination were measured. Litter also significantly affected the pre-vaccination bacterial agglutination titers and the post-challenge responses for lymphocyte blastogenesis, bacterial microagglutination and LPS hemagglutination. *Salmonella* were never detected in the feces of pigs for 8 days following vaccination. However, then the pigs were followed beyond the 8-day study period, *Salmonella* was isolated from the feces of vaccinated and nonvaccinated pigs. However,

vaccinated pigs shed *Salmonella* less frequently when compared to non-vaccinated pigs. The difference in fecal shedding was significant. Thus, this vaccine did not offer sufficient protection in pigs that would eliminate fecal shedding.

The desirable attributes of a safe, efficacious live attenuated *Salmonella* vaccine and some attenuated *Salmonella* mutants have been described (Curtiss et al. 1993). Three consequences of infection of a host by *Salmonella* were described: death of the host animal, the development of a carrier host animal, or the establishment of an adequate immune response that results in the eventual elimination of *Salmonella* from the host. The morbidity that is associated with infections from *Salmonella* was associated with the infecting *Salmonella* causing immune suppression thereby facilitating the establishment of a carrier state which might enhance the frequency of secondary infections and diminish the biological performance of the host as measured by feed conversion and weight gain. These investigators listed six attributes of a safe, efficacious, live vaccine as follows: 1) totally avirulent for both animals and humans, 2) highly immunogenic with induced immunity lasting until the animal goes to market and result in the blocking of *Salmonella* invasion of internal organs and reducing colonization of the intestinal tract by diverse *Salmonella*, 3) be genotypically stable with two or more attenuating deletion mutations, 4) have the attenuating phenotype unaffected by the diet or the host, 5) have the attenuating property of the avirulent *Salmonella* and not be dependent on fully functioning host defense mechanisms, and 6) be easy to grow, store, and administer. In addition, the vaccine strain would not be shed to animals not intended to be vaccinated nor should it persist in the environment. These

investigators also reported that two mutations of *S. typhimurium* (üD*cya* and D*cdt*) have been shown to be effective against experimental challenge by *S. typhimurium* and *S. choleraesuis*.

An experimental vaccine was developed and evaluated using a live avirulent *S. typhimurium* strain to protect immunized chickens against challenge with homologous and heterologous *Salmonella* serotypes (Hassan and Curtiss 1994). Heterologous strains used for the challenge of vaccinates were *S. agona, S. heidelberg, S. bredeny, S. albany, S. hadar, S. infantis, S. montivideo, S. enteritidis, S. panama,* and *S. anatum*. Group B challenge vaccinates had significantly lower organ colonization as did vaccinates challenged with those strains from serogroups D and E. There appeared to be a lower degree of protection in vaccinates that were challenged with strains from serogroup C. This suggests that protective immunity to *Salmonella* infection is related to LPS and that cross protection occurs between some serogroups and not others.

The safety, efficacy, and duration of immunity induced in swine vaccinated with an avirulent live *S. choleraesuis* vaccine (Ssc 54) was reported (Roof and Doitchinoff 1995). Intranasal administration of the vaccine did not produce untoward effects when vaccinates were compared with nonvaccinates for 14 days following vaccination. Vaccinates were later challenged with *S. choleraesuis* at 2, 8, or 20 weeks post vaccination. Clinical signs were significantly better in the vaccinates when compared to the nonvaccinates. Organ colonization in vaccinates was also significantly reduced compared to nonvaccinates. Pneumonia lesions were significantly reduced in the vaccinates. Weight gain tended to be better in the vaccinates but not significantly better. It was concluded that vaccination with

the avirulent vaccine was safe and protected pigs from experimental challenge with *S. choleraesuis* for at least 20 weeks following vaccination.

A commercially-available killed *S. typhimurium* vaccine approved for use in cattle was used in pigs that were subsequently challenged with *S. typhimurium* (Wingstrand et al. 1996). Two subcutaneous doses of the bacterin were given to the principals at 34 and 21 days prior to challenge. Control and vaccinates were challenged orally with 5 x 10^{10} CFU *S. typhimurium* PT12 Rif^R. Vaccinates and controls developed clinical signs characteristic of *S. typhimurium* infection and most pigs shed at 10^{12} CFU for at least one day. The duration of clinical signs in the vaccinates was significantly but modestly reduced compared to the nonvaccinates. There were no differences observed in diarrhea or rectal temperature. It was concluded that the bacterin did not protect pigs against challenge. In addition, vaccination induced seroconversion that was detected by the mix-ELISA. Thus, vaccination of pigs for *Salmonella* could pose a problem in *Salmonella* control programs that use the mix-ELISA as a herd screening tool for *Salmonella* infections.

Genetically engineered vaccines have not proved effective due to the loss of proteins, enzymes, and toxins relevant to the host-parasite relationship (Kramer 1995). This report conflicts with what was reported earlier in this section (Hassan and Curtiss 1994); Curtiss, 1993 #343). Perhaps the conflict can be explained by differences in vaccine efficacy observed in experimental vaccine trials compared to vaccine field trials with genetically engineered. For instance, the gut flora of conventional pigs may not support the growth of genetically engineered vaccine strains of *Salmonella*, precluding the development of a protective immune response.

Effect of infections on the biological performance of swine

General

An important swine management procedure is the movement of pigs into facilities in an all-in-all-out (AIAO) manner (Harris 1992). This means that pigs are placed into an empty, cleaned and sanitized room that is entirely emptied, cleaned, and disinfected before the next group of pigs is allowed to enter. This is in contrast to continuous flow (CF) pig management in which there is no time in which a room or building does not house pigs. The benefits of AIAO over CF include improved pig performance, reduced pathogen prevalence, and improved clinical appearance. In a two-year study to determine the effects of AIAO on the health of growing pigs (Scheidt et al. 1995) is was found that pigs raised in an AIAO manner performed better than pigs raised in a CF manner. Thirty-three pigs were raised in AIAO facilities and 33 pigs were raised in CF facilities. Six replicates of this study were conducted using the same source herd that had a history of clinical disease produced by *M. hyopneumoniae* and *P. multocida*. Each group of pigs was evaluated clinically prior to slaughter and sampled at the time of slaughter. Eighty-five of 198 CF (43%) exhibited some or all of the following clinical signs: dyspnea, gauntness with rough hair coat, lameness, and diarrhea. Fourteen of 198 AIAO pigs exhibited some or all of the following clinical signs: gauntness with rough hair coat, lameness, and ear lacerations. Five pigs in the CF group died during the study period compared to no deaths in the AIAO group. At slaughter, AIAO pigs had 54% less prevalent lung lesions and 80% less severe lung lesions compared to the CF pigs. *Mycoplasma hyopneumoniae* was detected in lung samples by indirect fluorescent

antibody in 8 of 10 lungs with lesions from CF lungs compared to 1 of 10 lungs from AIAO pigs. There were no bacteria cultured from the 10 AIAO lungs compared to 3 of 10 lungs from CF pigs that were positive on bacteriologic culture. Average daily gain for AIAO pigs was 1.74 compared to 1.54 for CF pigs.

The association between low levels of pathogens and improved biological performance has been demonstrated within commercial swine production systems (Harris et al. 1990). Animals were weaned at 8-10 days of age and moved to nursery facilities that were on a site separate from the farrowing facility. Control pigs were kept on the sow and weaned according to routine procedures for the farm. At 63-81 days of age, experimental and control pigs were weighed and necropsied. Pigs that were produced in the isolated nursery were significantly heavier than controls at the end of each experiment. Additionally, the thymus glands of the isolated weaned pigs were heavier than the thymus glands of the control pigs. There were no signs of clinical disease in any of the control or isolated weaned pigs. Atrophy of the thymus gland has been associated with exposure of the host to antigens, including, certainly, pathogens. These results suggest that exposure of swine to antigens that would be sufficient to cause thymus atrophy results in reduced biological performance compared to cohorts that are not so exposed.

Effect of swine management systems on pig health and performance.

Disease management in modern swine facilities includes the use of antimicrobials, vaccines, proper sanitation, and good husbandry practices. When these methods are not successful in controlling diseases that result in economic losses, other management strategies are often used. In the past, pork producers often eliminate economically significant disease

by the costly procedure of total depopulation, clean-up of facilities and repopulation with surgically-derived or minimal-disease breeding stock (Harris 1988). Unfortunately, this process is futile due to poor isolation of the herd from other high disease status pig herds.

In an effort to reduce the costs of depopulation and repopulation, a procedure known as medicated early weaning (MEW) was developed in order to produce pigs that are free of many pathogens that were endemic in the source herd (Alexander and Harris 1992). The MEW procedures use sows with at least one previous litter are bred in small groups at regular intervals. Sows are removed from the source herd at 110 days of gestation and placed in farrowing crates in an isolated farrowing facility. The isolated farrowing facility must have rooms that can be managed in an all-in-all-out manner. Sows are medicated immediately prior to leaving the source herd and for 5 days after farrowing. Sows are induced to farrow. Piglets are weaned at 5 days of age and transferred to an isolated nursery facility with rooms that can be managed in an all-in-all-out basis. After piglets are 5 to 8 weeks of age, they are again moved to grow-out facilities or to the recipient herd. This method of pig rearing requires the use 3 or 4 different facilities, each located in an area isolated from other swine herds.

Producing pigs by MEW was modified to decrease costs and allow for continued production of high health status breeding stock and slaughter pigs, from one or more source herds, without the removal of sows from the source herd (Harris 1988). Piglets were weaned at 10 days of age and place in a multiple isolated nursery facilities. The success of this system was attributed to being able to totally depopulate each isolated facility followed by repopulation with high-health piglets from farrowing. High-health status piglets could be

produced if piglets received adequate colostral antibody and/or medications prior to leaving the source farm. Inherent to the success of this system, called multi-site production, is a thorough knowledge of the diseases that have been targeted for elimination and strict adherence to AIAO. Such knowledge is important for the selection of vaccines or medications that would be used in the source herd. This method of rearing high-health status piglets is known as modified MEW (Isowean™) (Harris 1992).

Later, it was discovered that the principles of Isowean™ could be applied to commercial swine operations as well as for the production of breeding stock (Harris 1992). The need for adapting Isowean™ to commercial facilities arose from failure of traditional pig rearing systems to be able to eliminate endemic disease. Traditional pig rearing systems are usually of two types: farrow to finish (1 site) or farrow to feeder pig production with off-site finishing facilities (classic 2 site). One of the problems with 1 site or classic 2 site production is the frequent mixing of pigs from several farms in order to fill finishing facilities. Typically, this mixing occurred when pigs were 8-10 weeks of age. Pathogens from one source were then mixed with the pathogens of the other source farms, often with serious economic consequences for the producer. In order to accommodate the production from multiple source farms, the principles of Isowean™ are applied to the production system so that pig flow from the source farms can be coordinated at the time of weaning to fill multiple geographically isolated nurseries which in turn fill multiple geographically isolated finishing facilities (multiple isolated site or multi-site production) with piglets of the same age and health status. One benefit to multiple site production is the opportunity to increase the sow herd size of the source farms by 40%. In some instances, only one source herd might

be available to fill a nursery. In such instances, Isowean™ principles can still be applied by weaning piglets into an isolated nursery and then move them to isolated finishing production facilities. Thus, production occurs on three sites (3-site production).

Multi-site production has advantages over 3 site, classic 2 site, and 1-site production systems (Harris 1992). In multi-site systems, the production flow in the weaner and finisher stages remains continuous even in the presence of an infectious agent in the breeder/suckling stage. In the event that an infectious agent enters the weaner or finisher stages, the agent will be eliminated when the facility is emptied, following all-in-all-out procedures. This is in contrast to 3 site, classic two site or 1 site systems in which disease elimination might require depopulation and repopulation of the entire weaner and/or finisher populations.

Infectious agents that have been eliminated via Isowean™ include *Pasteurella. multocida, Mycoplasma hyopneumoniae, Actinobacillus pleuropneumoniae*, pseudorabies virus, transmissible gastroenteritis virus, *Serpulina hyodysenteriae, Hemophilus parasuis, Bordetella bronchiseptica*, porcine parvovirus, swine influenza virus, *Leptospira* spp (Glock and Harris 1993). It is necessary for health advisors to have a thorough knowledge of the infectious agents that are desired to be eliminated because different agents require that piglets be weaned at different ages (Glock and Harris 1993).

The exact mechanisms that are involved with improved pig performance associated with Isowean™ are not yet fully understood. One mechanism that may be involved is the effect of dust and endotoxin on the physiology of the pig (Crowe et al. 1996). Dust concentrations and total and respirable endotoxin were measured in two types of swine-rearing facilities: an isolated nursery and a nursery within a conventional (1 site) farm. Both

nurseries were managed in an AIAO manner. In three separate trials, dust concentrations were significantly higher in the 1-site nursery compared to the isolated nursery at 3, 5, and 6 weeks in the first trial and 3, 4, and 6 weeks in the third trial. There were no significant differences n dust concentrations in the second trial. Total endotoxin concentrations were significantly higher in the 1-site nursery compared to the isolated nursery for weeks 3, 5, and 7 in the first trial, weeks 2, 4, and 5 in the second trial, and weeks 2, 4, 5, 6, and 7 in trial 3. Respirable endotoxin was significantly higher in the 1-site nursery for weeks 5 and 7 in the first trial, week for 4 in the second trial, and weeks 4, 5, and 7 in the third trial. Necropsied pigs from the isolated nursery had significantly larger thymus glands (first trial), spleens (first and second trials), and cervical lymph nodes (second trial). Necropsied pigs from the 1-site nursery had significantly larger cervical lymph nodes in the first trial. Weights of pigs in the isolated nursery also tended to be higher than weights of pigs in the 1-site nursery. Although there were other confounding conditions that may also account for the differences observed, this preliminary study did suggest that there may be an association between airborne contaminants and pig performance.

The effect of immune challenge, dietary energy density, and source of energy on the performance and immunity of early weaned pigs has been reported (Heutgen et al. 1996). Pigs that received two injections of LPS were found to have reduced feed intake and reduced average daily gain. Feed efficiency was not affected by LPS injection. Increasing the energy density of the diet did not reduce the performance depression associated with the activation of the immune response of the pigs. There were no differences in biological performance or immune response in pigs fed with lard or corn starch as energy sources.

The affect of LPS-induced immune challenge on the growth performance of segregated early-weaned pigs has been reported (Dritz et al. 1996). Segregated early weaned (SEW) pigs were weaned at 14 days of age and placed into an isolated nursery. LPS-treated pigs were injected on days 5, 8, 11, and 14 postweaning. The study was terminated 32 days after weaning. Pigs that were not injected with LPS were significantly heavier than pigs that were injected with LPS. The growth differences were associated with decreased average daily feed intake and decreased feed efficiency. It was also found that LPS-injected pigs had higher serum haptoglobin concentrations. These results suggested that LPS does affect the physiology of the pig by stimulating the immune system which decreases growth by decreasing average daily feed intake and affecting the efficiency of nutrient utilization.

Effect of *Salmonella* infections on the biological performance of swine.

In a cohort study of farms that used whey and those that used water to add moisture to swine feed, farms that used whey had fewer samples that were positive for *Salmonella*. In addition, weight gains on the farms that fed whey and had lower *Salmonella* isolation rates from feces tended to be higher than those from farms that used water as a moisturizing ingredient to the swine feed (vanSchie and Overgoor 1987).

In a controlled study in which antibiotic feeding was evaluated for its ability to affect the duration of shedding and the clinical course of infection *by S. typhimurium*, biological performance of infected pigs was compared to the biological performance of non-infected pigs (Wilcock and Olander 1978). The study period of this experiment lasted 105-120 days. Average daily gain and feed efficiency were evaluated for four time periods post challenge with *S. typhimurium* on day 0-30, 31-60, 61-90, and 91-120. During the first 30 days of the

trial, average daily gain and feed efficiency were sharply reduced in infected pigs when compared to non-infected pigs. Performance parameters measured during the other 3 time periods were the same among all treatment groups. These results suggested that weight gain is impaired during the clinical period of the disease but not during the convalescent period.

The economics of salmonellosis in swine has been reviewed (Schwartz 1990). This review reported that results from a National Animal Health Monitoring Service survey that put the cost of salmonellosis at $2.3 million per month during the period of 1988-1989. Additionally, the economics of a salmonellosis outbreak were reported. Economic losses due to *Salmonella* infection were associated with death loss, medication costs, lost feed efficiency, extra facility costs and increased numbers of culls. In a group of 500 head that were placed on feed, costs totaled $6640 or $13.28 per head.

CHAPTER THREE. MATERIALS AND METHODS

Vaccine studies

Vaccine study number one. Vaccination of pigs at one day of age followed by challenge at 35 days of age with *S. choleraesuis*

Source of animals for vaccine study number 1

A swine herd known to be free of *S. choleraesuis* was selected as the source herd for pigs for this experiment. The herd was determined to be free of *S. choleraesuis* by virtue of no recent history of infection. Rectal swabs and blood samples from sows were collected prior to farrowing for bacteriologic examination and serologic examination for the presence of *Salmonella* or antibody to *Salmonella*. All sows were induced to farrow within 24 hours of each other. Fifteen litters farrowed within 24 hours of each other were used for this experiment.

Selection of animals and assignment to treatment groups for safety portion of vaccine study number 1

All sows farrowed within 24 h of each other were randomly assigned to one of two litter treatment groups: piglets vaccinated at one-day of age (L1, n=3) and piglets not vaccinated (L2, n=12).

Selection of animals and assignment to treatment groups for vaccine study number 1

Twenty pigs from L1 and 20 pigs from L2 were selected based on their physical appearance as judged to be able to be safely transported for 3-4 h to an isolation facility where the challenge study was conducted. The 20 pigs from L1 were randomly assigned to two rooms in the treatment facility and designated as treatment group 1 (Group 1).

Likewise, the 20 pigs from L2 were randomly assigned to two rooms in the isolation facility and designated treatment group 2 (Group 2, vaccinated at 21 days of age) and treatment group 3 (Group 3, not vaccinated).

Vaccination of pigs for vaccine study number 1

The vaccine was rehydrated using the accompanying diluent to achieve a concentration of 1 x 10^8 CFU per ml. Pigs from Group 1 were vaccinated intranasally (IN) with 2 ml into one nostril according to manufacturer's instructions within 24 h of birth. At 21 days of age pigs from Group 2 were similarly vaccinated IN. Pigs from Group 3 were not vaccinated.

Bacteriologic examination of sow rectal swabs for vaccine study number 1

Rectal swabs collected from sows were transferred to Rappaport-Vassiliadis (RV) (Difco Laboratories, Detroit, MI) broth and incubated at 42 °C for 24 hours. After incubation, RV tubes were vortexed and streaked onto xylose lysine dehydrogenase (XLD, Dimed Corporation, St. Paul, MN) agar. XLD plates were incubated overnight at 37°C. Suspect *Salmonella* colonies were transferred to Kligler's iron agar (Dimed Corporation, St. Paul, MN), trypticase soy agar (Dimed Corporation, St. Paul, MN), and urea agar slants (Dimed Corporation, St. Paul, MN) and into sulfur-indole-motility (SIM) semisolid medium (Dimed Corporation, St. Paul, MN). All media were incubated at 37°C for 18-24 h. Presumptive *Salmonella* colonies were agglutinated with polyvalent O antigen antisera (Difco Laboratories, Detroit, MI) to confirm *Salmonella* genus. Presumptive *Salmonella* isolates were submitted to the National Veterinary Services Laboratory (NVSL), Ames, IA, for confirmation and serotyping.

Monitoring of pigs used for vaccine study number 1 prior to weaning

Following vaccination and prior to weaning, all pigs in groups L1 and L2 were observed daily for 7 days. Clinical signs that were measured were stool consistency, behavior, appetite, body condition, neuromuscular signs, arthrologic signs, ambulation, hair coat, skin condition, and pneumonia. A subjective score was given to each clinical score parameter (range 1-4; 1=normal and 4=dead). Table 1 lists the scoring method used in this study. Clinical scores for pigs that died were maintained throughout the 7 day observation period. At the end of each day, all clinical parameters for each pig were added to determine a daily clinical score. A score of 10 was considered normal and a score of 40 indicated that the pig had died.

Table 1. Scoring system used for determining clinical score for pigs for vaccine studies.

	Score			
Clinical Parameter	**1**	**2**	**3**	**4**
Stool Consistency	Firm	Semi-formed	Diarrhea	Dead
Behavior	Active/Alert	Lethargic	Huddled	Dead
Appetite	Normal	Diminished	Anorexic	Dead
Body Condition	Full abdomen	Gaunt	Thin	Dead
Neuromuscular	Normal	Head tilt/Circling	Tremors/Seizures	Dead
Arthrologic	Normal	Joint Swelling	Non-use	Dead
Ambulation	Normal	Lame	Recumbent/down	Dead
Hair Coat	Smooth	Rough	Bristled	Dead
Skin	Normal	Urticaria	Cyanosis	Dead
Pulmonary	Normal	Panting	Dyspnea	Dead

Monitoring and care of pigs used for vaccine study number 1 after transport

After movement to the isolation facility and assignment to treatments and rooms, pigs were fed a non-medicated commercial diet twice each day and provided free-choice water and supplemental heat.

When pigs were 28 days old, clinical scores were recorded daily as described on page 64 for seven days prior to challenge and daily for the 14 days after challenge. Rectal temperatures were also recorded daily for seven days prior to challenge and for each day after challenge. Pigs that died during the course of the experiment were weighed and necropsied. All surviving pigs were euthanized and weighed at 48 days of age. Blood samples were collected from all pigs at 27 days of age and at the end of the study.

Challenge of pigs used for vaccine study number 1

At 35 days of age (Day 0), each pig was weighed and received an IN challenge dose of 1×10^{10} *S. choleraesuis* isolate χ-3246. This isolate originated from a clinical outbreak of porcine paratyphoid and was obtained from Dr. Roy Curtiss (Washington University, St. Louis, MO) and was prepared in brain heart infusion (BHI) broth as previously described (Roof and Doitchinoff 1995).

Necropsy of pigs used for vaccine study number 1

Each pig in this study was necropsied following death during the study or at the termination of the study. Samples were collected from the tonsil, lung, liver, spleen, mesenteric lymph node, ileum, and colon. A pneumonia score was determined for each pig. This score was an estimate based on the subjective visual assessment of the percent of lung that showed lesions of pneumonia.

Bacteriologic examination of samples collected for vaccine study number 1

Organ samples were cultured for the presence of *Salmonella* as previously described (Roof and Doitchinoff 1995). Briefly, organ samples collected at necropsy were trimmed to a weight of approximately 1 g, weighed, homogenized and transferred to buffered peptone water (BPW, Difco, Detroit, MI) and incubated at 37 °C for 18-24 h. Cultures were vortexed and 100 µl of each sample was transferred to Rappaport-Vassiliadis (RV) broth and incubated at 42 °C for 24 h and processed as described above. The number of *Salmonella* per gram of tissue sample was calculated.

Serologic examination of samples collected from pigs for vaccine study number 1

Blood samples were centrifuged to separate serum from cells. Serum was transferred via pipette to plastic storage vials, capped and stored at -70 °C . Serum samples were shipped frozen and packed in ice prior to submission to the Danish Veterinary Laboratory, Copenhagen, Denmark. The level of antibody to *Salmonella* was measured using the Danish mix-ELISA as previously described (Nielsen et al. 1995). *Salmonella*-specific antibodies in serum were detected by an indirect enzyme-linked immunosorbent assay (ELISA) that uses lipopolysaccharide (LPS) from *S. choleraesuis* and *S. typhimurium* as the coating antigen (Nielsen et al. 1995). This test is referred to as mix-ELISA. Briefly, Polysorp (Nunc, Denmark) microwell plates were coated with 100 µl of a solution of LPS in coating buffer (0.1 M sodium carbonate, 1.0 M NaCl, pH 9.6), sealed with plastic, and incubated overnight at 5 °C. The plates were emptied and blocked for 15 min and then washed. The washing buffer used in these tests was phosphate-buffered saline (PBS) containing 0.05% Tween 20 (PBS-T). Bovine serum albumin (BSA, Sigma) was added (1% w/v) to the buffer (PBS-T-

BSA) for blocking the plates and for dilution of sera and conjugates. The test and control sera were diluted 1:400 in PBS-T-BSA and applied in duplicate and incubated for 1 h at room temperature, followed by three cycles of washing. Horse radish peroxidase labeled rabbit antiserum to porcine immunoglobulin (Ig) was diluted 1:2000 in PBS-T-BSA and 100 µl was added to each well. The conjugate was incubated for 1 h at room temperature and the microwells were washed as before. Then, 100 µl of substrate was added (5 µl H_2O_2, 30%, 8 mg of 1,2 orthophenylenediamine dihydrochloride, 12 ml 0.1 M citrate, pH 5). After 10 min. incubation, the color development was stopped by adding 100 µl of 0.5 M H_2SO_4. The optical density (OD) was read at 490 nm with background correction of 650 nm using a Molecular Devices "Emax" plate reader.

Sample OD's were transformed to calibrated OD's using a linear regression equation for reference OD's on the actual plate versus mean reference OD's. Finally, each sample measurement was expressed as an OD% using the following equation:

OD% = ((Calibrated Sample OD - 0.176)/(3.068 - .176)) * 100

where 0.167 was the mean OD of the negative reference serum and 3.068 was the mean OD of the positive reference serum.

Results from optical density readings were reported as OD%. Mix-ELISA results were reported as a calculated OD%. OD% greater than 10 was determined to be positive.

Statistical analysis of data collected for vaccine study number 1

Analysis of variance, and general linear models and means contrasts and estimates were used to analyze data and were performed by commercially-available statistics software (SPSS 1997); (SAS 1985).

Vaccine studies two and three. Vaccination of pigs at 21 and 1- day of age followed by challenge with *S. typhimurium*.

Source of animals for vaccine studies two and three

In vaccine studies two and three, two different swine herds free of *Salmonella* were selected as the source herds for pigs for this experiment. These herds had no history of clinical *Salmonella* infections One herd was the same herd used as a source of pigs for the first experiment and a second herd was used as a source of pigs for the second experiment. Rectal swabs and blood samples from sows were collected prior to farrowing. All sows were induced to farrow within 24 hours of each other. Litters that farrowed within 24 hours of each other were used for this experiment.

Selection of animals and assignment to treatment groups for vaccine challenge studies two and three

In vaccine study number two, 30 pigs were chosen from 6 litters, weaned at 19 days of age and transferred to isolation facilities. Upon arrival at the isolation facilities all 30 pigs were individually ear-tagged. Twenty (20) pigs were randomly assigned to Treatment Group 1 (S1G1, vaccinated; challenged with *S. typhimurium*) and 10 pigs were randomly assigned to Treatment Group 2 (S1G2; not vaccinated; challenged with *S. typhimurium*).

In vaccine study number three, ten litters of pigs were farrowed within 24 h of each other and each litter was randomly assigned to one of two treatment groups, vaccinated (n=5) or not vaccinated (n=5). Prior to weaning, 15 pigs were randomly selected from litters that were not vaccinated and assigned to one of two nonvaccinated treatment groups: Treatment group 1, nonvaccinated, not challenged (n=5) and Treatment Group 3, nonvaccinated, challenged with *S. typhimurium* (n=10). Twenty-five pigs were randomly selected from the

litters that were vaccinated and assigned to one of two vaccinated treatment groups: Treatment Group 2, vaccinated but not challenged (n=5) and Treatment Group 4, vaccinated and challenged with *S. typhimurium* (n=20).

Vaccination of pigs for the vaccine challenge studies two and three

Pigs used in vaccine study number two were vaccinated IN at 21 days of age as previously described. Pigs used in vaccine study number three were vaccinated IN within one day of age as previously described on page 62

Monitoring of pigs used in the vaccine studies two and three prior to weaning

Following vaccination and prior to weaning, all pigs in study two were observed for 14 days as previously described on page 63.

Monitoring and care of pigs used in the vaccine studies two and three after transport to isolation facilities

After movement to the isolation facility and assignment to treatments and rooms, pigs were fed a non-medicated commercial diet twice each day, provided free-choice water and supplemental heat. When pigs were 28 days old, all pigs were weighed and clinical scores were recorded daily prior to challenge and for each day of the study as described above. Pigs in vaccine study number two were observed for 7 days prior to challenge. Pigs in vaccine study number three were observed for 3 days prior to challenge. Rectal temperatures were also recorded daily prior to challenge and for each day of the study for each pig. Pigs that died during the course of the experiment were weighed and necropsied. All pigs that survived until the end of the study period were euthanized and weighed at 35 days of age. Blood samples were collected from all pigs at 27 days of age and at the end of the study.

Challenge of pigs used in vaccine studies two and three

Pigs in vaccine study number two received their challenge dose (7.5×10^9) of *S. typhimurium*, strain 102 (NVSL Federal Reference) at 35 days of age. Pigs in vaccine study number three received their challenge dose of *S. typhimurium* at 28 days of age. Both challenge strains were prepared as previously described for *S. choleraesuis*.

Necropsy of pigs in the vaccine studies two and three

Each pig in vaccine study two and three were necropsied following death during the study or at the termination of the study. Organ samples were collected from the tonsil, lung, liver, spleen, mesenteric lymph node, ileum, and colon as previously described. A pneumonia score was determined for each pig as previously described herein.

Bacteriologic examination of samples collected during the vaccine studies two and three

Organ samples collected at necropsy were cultured for *Salmonella* as previously described herein.

Serologic examination of samples collected during vaccine studies two and three

Blood samples collected from pigs in vaccine studies two and three were handled, stored, and submitted for testing as previously described herein.

Statistical analysis of data from vaccine studies two and three

Analysis of variance, and general linear models and means contrasts and estimates were used to analyze data and were performed by commercially available software as previously described. Odds likelihood calculations were done by hand (Thrusfield 1995).

Vaccine study number four. Bacteriologic and serologic comparison of vaccinated and nonvaccinated pigs in a commercial farm with a history of *Salmonella* infections

Farm used for source of animals in vaccine study number four

A farm located in the Midwest with a history of clinical *Salmonella* infections was used in this study. *Salmonella choleraesuis, S. typhimurium, S. heidelberg, S. infantis,* and *S. derby* were among the serotypes isolated from this farm's pigs within the past year (ref. Tables 26 and 43). This was the same farm as Farm C that was used in the epidemiologic studies reported later in this dissertation. Outbreaks of salmonellosis occurred at various stages during the finishing period. The farm management team agreed to participate in a field vaccine study (D. Polson, J. Kolb, personal communication) whereby one-half of the pigs produced by the farm within a 6 week period of time would be vaccinated with SC54 ™ . The original purpose of this study was to demonstrate the efficacy of the vaccine for the protection of pigs against clinical *Salmonella* infections on this farm. Following the completion of the field efficacy study, the farm management team agreed to allow for the collection of samples from some pigs from each treatment group after they had been slaughtered.

Experimental design of vaccine study number four

This study was designed as a case-control study. A case was defined as a finishing building in which pigs had been vaccinated via the drinking water 14 days after placement. A control was defined as a finishing building in which pigs had not been vaccinated. There were 5 complexes of finishing buildings from which pigs were available for sample collection. Each of these complexes contained 8 confinement finishing buildings that had

been randomly assigned to two treatment groups: vaccinated or nonvaccinated. Each finishing building housed approximately 1,200 head of swine. A finishing building had been considered to be the experimental unit for the vaccine efficacy study and was used as the experimental unit for this study. There would be about 50 samples from 20 groups of nonvaccinated pigs and 20 groups of vaccinated pigs. Thus, the total samples for each treatment group would be about 1,000. This sample size would allow for the detection of a significant difference if prevalence in one group of pigs was 18% and 13% in the other group of pigs (Martin et al. 1987).

Selection and handling of swine prior to slaughter for vaccine study number four

All pigs that were removed from the finishing buildings of each of the locations had been in the building for about the same length of time (110-114 days). Pigs were removed from the buildings and sent to slaughter according to the usual management practices of the farm. After arrival at the slaughter facility, all animals were rested for at least 90 minutes before being killed. After entering the killing area, it took an additional 40 minutes for the pigs to arrive at the area of the slaughter facility where samples were collected.

Sample collection from pigs used in vaccine study number four

Ileocecal lymph nodes and a sample of diaphragmatic muscle (approximately 3 cm^3) were collected from each carcass. Each carcass was identified individually and by the building from which it was taken.

Efforts were made to collect the lymph nodes with aseptic technique, given the speed of carcass movement while samples were being collected. The gloved hands of the collector

and the knife used to collect the lymph nodes were rinsed in hot (approximately 140° F) water between each carcass.

Meat and lymph node samples were place into sterile bags, sealed and put in coolers with ice for transportation to the laboratory.

Handling and storage of samples from vaccine study number four

Meat samples were frozen at -70 °C while the lymph nodes were held at 4 °C prior to processing. Lymph nodes were cultured on the day following collection.

Bacteriologic examination of lymph nodes for vaccine study number four

All mesenteric lymph nodes were macerated in their sample bag. Approximately 1 gram of sample was transferred into buffered peptone water (BPW), vortexed and incubated at 37 °C for 18-24 as described previously.

Fifty-six samples were randomly selected from the last day that samples were collected. These samples were split into two different portions and cultured by two methods to determine if both methods were equally capable of detecting groups of pigs with high levels of *Salmonella.* One portion was cultured in the same manner as previously described (Roof and Doitchinoff 1995). The second portion was cultured according to the procedures described in epidemiologic study number (Fedorka-Cray et al. 1995).

Serologic examination of juice from meat samples for vaccine study number four

All meat samples were thawed at room temperature in their respective collection bag several days after collection. Immediately after thawing, juice that was expressed from the meat sample was drawn from the bag with a pipette and transferred into micro-centrifuge tube and frozen for analysis later. Almost all of the meat juice samples were cloudy and

contained debris. Those samples with debris were centrifuged in an effort to remove the debris. The meat juice samples were frozen at -70 °C for storage until they were tested by NOBL Laboratories for analysis by the mix-ELISA.

Thawed meat juice samples were diluted 1:30 (Nielsen et al. 1996) prior to testing in the mix-ELISA. The mix-ELISA (Nielsen et al. 1995) was performed as previously described on pages 65 - 66.

Statistical analysis of data collected from samples for vaccine study number four

Analysis of variance for comparing means from the two treatment groups was performed using commercially available statistics software as previously described on page 69. The culture prevalence, serologic prevalence, and the mean OD% of the two treatment groups were compared. In addition, the differences in prevalence of the isolated serotypes of *Salmonella* were compared between the two treatment groups.

Vaccine study number five. Serologic response of pigs vaccinated at 21 days of age

Source and treatment of pigs for serologic examination of vaccine study number five

Ninety-eight 21-day old pigs from a commercial farm with a history of *S. choleraesuis* were individually tagged, bled, and divided into two treatment groups on the basis of the pens in which they were housed. There were four pens from which pigs were used for this portion of the study. One group (n=50) was vaccinated with SC54™ according to label directions and kept in separate pens from the other group of (n=48) non-vaccinated pigs. Pigs remained in their respective pens for approximately 6 weeks prior to being moved into a finishing building with totally slatted flooring and natural ventilation. The two

different treatment groups were not commingled at any time after movement into the finishing building.

Fresh feed and water were offered to all pigs via self-feeders and self-waterers throughout the course of this study.

Sample collection for vaccine study number five

Blood samples were collected from all pigs at the time of vaccination. Blood samples were also collected 90 days after vaccination from 30 randomly selected pigs from each treatment group. Serum from each blood sample was removed from each blood clot and stored at -70 °C until they were test in the mix-ELISA.

Serologic examination of samples collected for vaccine study number five

All serum samples were tested using the mix-ELISA (Nielsen et al. 1995) as previously described on pages 65-66.

Epidemiologic studies

Epidemiologic study number one. Serologic and bacteriologic studies of blood and fecal samples collected from three farms

Study design

A serial cross-sectional study was conducted on three commercial farms in the upper Midwest. Monthly visits were made to each farm for the purpose of sample collection. Farm A was visited during 12 months of the period from December, 1994, through December, 1995. Farm B was visited monthly for the period of January, 1995, through December, 1995. Farm C was visited monthly for the period of December, 1994, through December, 1995. There were no samples collected from Farm C during the month of July, 1995. An

experimental unit was defined as a building or set of buildings that housed a group of pigs that was within two weeks of slaughter at the time of the farm visit.

Description of farms used in this study

The three farms were designated Farm A, Farm B, and Farm C. Each of these farms had swine finishing facilities for the purpose of growing and finishing swine to market weight, about 240 pounds. Each of these farms production type was differentiated on the basis of whether or not an isolated nursery was used by the farms. An isolated nursery was defined as a nursery that was physically separated from the breeding herd and from any portion of the finishing herd population. In addition, an isolated nursery was managed in an all-in-all-out manner. Pigs that were placed into the nursery were farrowed within one week of each other

All three farms purchased their breeding stock from breeding stock companies. Most of the breeding stock for all three farms was purchased from the same company.

Farm A was a 250-sow farrow-to-finish swine farm with facilities located on two separate sites, separated by approximately one-half mile. One site contained outdoor breeding housing, outdoor gestation housing, one confined farrowing building and one confined, partially-slatted, power-ventilated finishing building. The second site contained a farrowing building, gilt isolation, nursery, and three outdoor finishing buildings. Thus, this farm did not have an isolated nursery. This farm produced about 4,500 head of market swine per year. Swine were finished in one of four finishing buildings: a partial slatted building, a cattle shed with solid flooring, a converted chicken house, and a converted dairy barn with solid flooring. Each of these facilities housed approximately 450 head of swine in 24, 4, 4,

and 5 pens, respectively. There was no previous history of *Salmonella* infection in the pigs of Farm A.

Farm B was a 1,000-sow farrow-to-finish swine farm. This farm had a history of *S. choleraesuis* infection in the year prior to the start of this study. The farm had been vaccinating pigs with a commercially-available avirulent, live *S. choleraesuis* vaccine for 3 months prior to the start of this study. This practice was discontinued at the beginning of this study. The decision to quit vaccinating pigs was made solely by the owner of the farm (D. Fisher, personal communication). This farm started the study as a classic 2-site swine production farm in which the breeding herd, farrowing facilities, and nursery facilities were located on the same site. Pigs were removed from this site at approximately 9 weeks of age and taken to one of three sites that contained finishing facilities. A new nursery had been constructed on a site that was approximately three-fourths of a mile from the breeding, gestation, and farrowing facilities. Thus, Farm B became a 3-site production system. Pigs that were tested in January and February were produced when the farm's production was 2-site. Pigs that were tested after February were produced when the farm's production was 3-site. There were two finishing facilities that housed tested pigs; one was a confined, totally slatted, naturally-ventilated building divided into two rooms that each housed 450 head of swine in 24 pens and the other was a finishing building that housed pigs that was an outdoor finishing building with an open concrete apron and shelter on the north side. This latter facility type is also known as a Cargill building. There were ten pens in this facility, each of which housed 30-40 head at a time. The nursery and both of the finishing facilities that housed pigs for this portion of the study were managed in an all-in-all-out manner.

Farm C was an 80,000-sow farrow-to-finish commercial swine production farm. There had been outbreaks of *Salmonella* infections in various groups of pigs in the year prior to the start of this study. *Salmonella typhimurium* and *S. choleraesuis* were the serotypes that had been isolated from previous outbreaks of salmonellosis. This farm was considered to be a classic two-site production system. Breeding, gestating, farrowing, and nursery facilities were maintained on one site. Pigs were moved from the breeding/suckling/nursery site at approximately 9 weeks of age and transferred to finishing facilities located on separate sites. All finishing facility sites had 8 buildings each of which housed approximately 1250 head of swine in 48 pens. The finishing buildings from which samples were collected for this portion of the study were totally slatted, naturally ventilated confinement buildings. Manure was removed by flushing recycled lagoon water under the slatted portions of the building. Each building contained pigs of one sex and were filled with pigs from as many as 4 different breeding/suckling/nursery facilities. Pig flow management was described as all-in-all-out for each finishing building within a finishing site.

Sampling procedures

At least 30 blood samples and 30 individual fecal samples were collected from the floors of the finishing facilities from each farm. A sample size of 30 allowed for detection of a farm prevalence of 10% with 95% confidence (Thrusfield 1995).

Because there were fewer than 30 pens in the facilities of Farms A and B, multiple individual fecal samples were collected from the floor of each pen. Blood samples were collected from multiple pigs from each pen from the finishing facilities of Farms A and B.

For each finishing site belonging to Farm C there were 8 buildings with 48 pens per building for a total of 384 pens for each experimental unit. Therefore, 30 pens were randomly selected from the 8 buildings for environmental fecal and blood sampling. Each set of eight finishing buildings in Farm C was considered an experimental unit.

Sample collection methods

Approximately 25 g of feces were collected from pen floors. A wooden tongue depressor was used to collect the feces and transfer the sample to a sterile, plastic 50-ml centrifuge tube. Care was taken to avoid touching the pen floor with the wooden tongue depressor. Samples were transported in ice coolers to the laboratory where they were refrigerated overnight before processing for bacteriologic culture.

Blood samples were drawn from a pig or pigs within the same pen that fecal samples were collected.

Blood and fecal samples were identified according to the building and pen from which they were collected.

Bacteriologic examination of environmental pen fecal samples

Approximately 5 grams of feces was transferred into GN Hajna (GN) (Difco) broth and tetrathionate (TET) broth (Difco) and incubated for 24 h and 48 h, respectively, at 37 °C. Following incubation of GN and TET, approximately 100 µl of each broth was transferred into Rappaport-Vassiliadis (R10) broth and incubated at 37 °C for 24 h. After incubation of the R10 broth cultures, cultures were streaked onto xylose lysine tergitol (XLT-4) agar (Difco) and brilliant green with sulfadiazine (BGS) (Difco) agar. Presumptive positive *Salmonella* colonies were inoculated onto lysine iron agar, triple sugar agar slants, and

trypticase soy agar slants. Further confirmation of *Salmonella* genus was made by agglutination of suspect colonies from the agar slants using polyvalent O antiserum (Difco), and *Salmonella* serogroup B, C1, D, and E antisera (Difco). Positive isolates were submitted to the National Veterinary Services Laboratory for serotyping. Results were reported as positive or negative. Positive results were further identified by serotype.

ELISA testing of serum

Serum samples were submitted to the Danish Veterinary Laboratory for testing in the mix-ELISA as previously described (Nielsen et al. 1995) on pages 65 - 66.

Interpretation of ELISA results

ELISA results were reported as OD% as described above. An OD% greater than 10 was considered positive. This cut-off was used to determine "scientific" *Salmonella* seroprevalence (Nielsen, personal communication). Samples were also evaluated using an OD% greater than 40 as the positive cut-off value for *Salmonella* antibodies. This cutoff was used to determine the "commercial" *Salmonella* seroprevalence. The "commercial" *Salmonella* seroprevalence was used to assign of groups of pigs to one of three *Salmonella* levels: 1, 2, or 3 (Nielsen et al. 1995). *Salmonella* level 1 groups had a seroprevalence of 10% or less, level 2, greater than 10% but less than 15% seroprevalence, and level 3, seroprevalence of 15% or greater.

Statistical analysis of results

Results from mix-ELISA testing of serum samples and bacteriologic examination of environmental fecal samples were analyzed using commercially available statistics software as previously described on page 65 - 66. Tests of analysis of variance were conducted to

determine the differences in *Salmonella* prevalence for each farm, each season of sample collection, and each production system type. Differences in prevalence were considered significant if the p-value was less than or equal to 0.05. *Salmonella* prevalence was determined from bacteriologic and serologic results. Then, sample differences in serologic results were compared between bacteriologic-positive samples and bacteriologic-negative samples to determine if a significant difference in seroprevalence existed. Finally, each group of pigs was classified as positive or negative by culture and the serologic results from mix-ELISA were compared.

Epidemiologic study number two. Serologic and bacteriologic studies of meat juice and mesenteric lymph nodes collected from pigs at slaughter from four farms

Study design for epidemiologic study number two

A serial cross-sectional study of *Salmonella* prevalence among groups of pigs was conducted from samples collected at the time of slaughter from swine produced on four farms. Samples of mesenteric lymph node and muscle were collected from pigs during the slaughter process. Mesenteric lymph nodes were collected for cultured for the presence of *Salmonella*. Muscle samples were frozen and then thawed to allow for collection of meat juice which was assayed for antibody to *Salmonella* using the mix-ELISA.

Null hypothesis for epidemiologic study number two

There is no association between the serologic prevalence of *Salmonella* antibodies in a group of pigs at slaughter and the culture prevalence of *Salmonella* in the same group of pigs.

Alternate hypothesis for epidemiologic study number two

There is an association between the serologic prevalence of *Salmonella* antibodies in a group of pigs at slaughter and the culture prevalence of *Salmonella* in the same group of pigs.

Sources of swine used for epidemiologic study number two

Farms A, B, and C from epidemiologic study number 1 sold pigs to slaughter for use in this study. In addition, a fourth farm, Farm D, provided samples from pigs sold into slaughter. Farm D had production facilities throughout the United States. The pigs provided for this study came from a new herd and new facilities in the Southwestern portion of the United Sates. Farm D production is known as "multi-site". Facilities consisted of separate sites for each of the following portions of the herd: breeding, gestating, and farrowing; nursery; and finishing. Pig flow for Farm D was managed in an all-in all-out manner with buildings being filled weekly with pigs that were born within one week of each other. Farm D had no previous history of *Salmonella* infections.

Sample collection for epidemiologic study number two

For Farms A and B, samples of mesenteric lymph nodes and muscle were collected at different times of the slaughter process. Viscera were collected from the kill line viscera trays while muscle samples were collected from carcasses after they were put into refrigeration. Thus, individual identification of samples was not possible. Therefore, samples were identified only to Farm A or Farm B by the date on which they were collected. This method of collection was necessary because of the rapid speed at which carcasses were processed. Mesenteric lymph nodes were collected after viscera from all pigs in the group

had been collected into a common plastic tub. Sterile scissors and forceps were used to collect each mesenteric lymph node. Muscle samples were then collected while carcasses were hanging in the carcass chilling refrigerator. Efforts were made to collect up to 30 samples from each farm. Facility breakdowns at the slaughter plant resulted in the cancellation of two collection times. Also, the owner of Farm B decided to quit participating in the project after the fourth collection. This decision was based on the lack of cooperation from the packer, plant shutdowns, and differences in prices received from the packer compared to another packer that usually purchased pigs from Farm B.

Samples from Farm C pigs were collected as described for vaccine study number 4. At least 30 samples were collected from each group of pigs from Farm C.

After analysis of data from Farms A, B and C, it was determined that additional samples were needed from another farm that had no history of *Salmonella* infections. One year after slaughter collections for Farms A, B, and C began, samples from Farm D were collected at slaughter by the veterinarian who routinely performed health checks at slaughter. Samples were collected from October, 1996, through December, 1996. Fifteen mesenteric lymph nodes and 15 muscle samples were collected from the same carcass and were identified to specific site and to individual animal. Samples of mesenteric lymph node and muscle collected and placed into their own sterile plastic bag, packed in dry ice and shipped to the laboratory for further processing.

Bacteriologic examination of mesenteric lymph nodes for epidemiologic study number two

Mesenteric lymph nodes collected from pigs from Farms A, B, and C were cultured using the method previously described on page 78. Mesenteric lymph nodes collected from pigs from Farm D were cultured using another method previously described herein.

Serologic examination of muscle juice for epidemiologic study number two

All muscle juice samples from Farms A, B, C, and D were tested for the presence of antibody to *Salmonella* using the mix ELISA as previously described on page 78.

Statistical analysis of data collected from epidemiologic study number two

Data collected were analyzed using commercially available statistics software as previously described page 69.

Epidemiologic study number three. Serologic survey and management survey of commercial swine farms

Study design for epidemiologic study number three

This was a serial cross-sectional study of the seroprevalence of *Salmonella* on farms in the United States.

Sources of pigs for sample collection for epidemiologic study number three

Farms were chosen for this part of this study based on their willingness to have samples submitted. A farm was designated a farm according to its location of day-to-day management. Farms A, B, C, and D from the previous epidemiologic studies were included in this study. Six other farms, Farms E, F, G, H, I and J were added to the other 4 farms for this study.

Farm E had approximately 10,000 sows and was owned by the same owner as Farm D but was located in a Midwestern section of the United States on several locations. Production of swine in Farm E was done in confinement facilities where the breeding, gestating, farrowing, and nursery facilities were located on one site while confinement finishing was located on separate sites or even on the same site as the rest of the herd. There were no isolated nurseries used for swine produced by Farm E. Thus, this farm production is known as classic 2-site.

Farm F had approximately 17,500 sows and was owned by the same owner as Farm C but was located in the Southwestern portion of the United States. This farm's production is known as multi-site. The breeding, gestating, and farrowing facilities were power-ventilated confined facilities located on one site. Nursery facilities were located on a second site and determined to be isolated. Finishing buildings were located on a third site. All nursery and finishing facilities were filled within one week of each other with pigs that were farrowed within one week of each other.

Farm G had approximately 1,000 sows and was located in Northern Iowa. Breeding, gestating, and farrowing facilities were confinement type and located on one site. This farm's production type is known as 3-site. The nursery that served this farm was located approximately one-half mile from the breeding herd and was considered to be isolated. Finishing facilities were also confinement type and were located at 4 different sites. The nursery was managed so that pigs could be flowed through in an all-in-all-out basis. Finishing facilities tended to be managed so that pigs were flowed in a continuous fashion.

Farm H had about 17,500 sows and was located in the Western portion of the United States. This farm's production is known as classic 2-site. Breeding, gestation, farrowing, and nursery facilities were of the confinement type and were located on one site. Finishing facilities were located on separate sites. Each nursery and each finishing building was filled with pigs that were farrowed within one week of each other. Nursery and finishing facilities were managed so that pigs were flowed into and out of the building in an all-in-all-out fashion.

Farm I was a testing station that finished pigs from an isolated nursery project conducted for another experiment. These pigs were thus produced in a 3-site manner

Farm J was a 500-sow farrow-to-finish farm in which one group of pigs was bled as part of another experiment. This farm's production is known as classic two-site. Thus, this farm did not have an isolated nursery.

Farm SC was a group of 70 pigs that were used for vaccine study number 1. These pigs were reared in an isolated nursery. These pigs were bled and tested for *Salmonella Y. enterocolitica* O:3 antibodies by ELISA.

Samples collected for epidemiologic study number three

Serum or meat juice samples were collected from groups of pigs produced from the 8 different farms. The method of sample collection from Farms A, B, and C have already been described herein. Also, the collection of some samples from Farm D have been described herein. In addition, serum samples from various groups of finishing pigs from Farm D were collected monthly by the herd veterinarian responsible for health decisions of the farm.

Serum samples from farm D were collected beginning in August of 1995 and concluded in December of 1995.

Serum samples from Farms E, F, G, and H were collected in the same manner as samples from Farm D.

Serum samples from Farms I and J were collected only once. Farm I was sampled in November, 1994, while Farm J was sampled in March, 1995.

Serum samples from Farm F ceased in June, 1995, but were replaced with collection of muscle samples at a slaughter plant by slaughter plant personnel for the period of August, 1995, through December, 1995.

Serologic examination of samples collected for epidemiologic study number three

All serum and meat juice samples were submitted to the Danish Veterinary Laboratory. The mix-ELISA was used to test all serum and meat juice samples for the presence of antibody to *Salmonella* as described previously.

In addition, serum samples collected from Farms C, D, E, and F were tested in the Danish Veterinary Laboratory for the presence of antibody to *T. gondii* (Dubey et al. 1995, T. spiralis (Lind, 1995 #796), and *Y. enterocolitica* O:3 (Nielsen et al. 1996).

Questionnaire survey of herds for epidemiologic study number three

A questionnaire (Table 2) was completed for each group of pigs that was sampled for this study and of consisted of 6 sections. The first section asked for information about pig and feed weights, dates in and out, culls and deads, and number of days on feed. The second section contained questions about the health status of the source farm and the group of pigs for which the questionnaire was completed. A third section asked for information about site

Table 2. Questionnaire used to collect information from groups of pigs used in epidemiologic studies.

FINISHER SITE:

DATE:

DATE FILL BEGUN:
DATE FILL COMPLETED:
DATE EMPTIED:
NUMBER OF HEAD IN:
TOTAL WEIGHT IN:
TOTAL DEATHS:
TOTAL CULLS:
NO. SOURCES:
TOTAL FEED IN:
TOTAL WEIGHT OUT
AVERAGE DAYS IN:

NO. BUILDINGS

BUILDING DIMENSIONS (OUTSIDE):

PIG FLOW: CONT AIA0-PEN AIA0ROOM AIAO-BLD AIAOSITE

FARM TYPE: MULTI-SITE 3-SITE SITE 2-SITE TRAD 2-SITE UNTRAD 1-SITE

GROW/FINISH DESIGN CONNECTED/SEPARATE/FINISHER ONLY

FEEDING SYSTEM DRY WET WET/DRY

BAIT STATIONS IN PLACE YES NO

IF IN PLACE, NUMBER: NO. DEAD RODENTS

VENTILATION: NATURAL POWER

MANURE REMOVAL: PIT FLUSH SCRAPE

FLUSH WATER: FRESH RECYCE

FLOOR TYPE: SOLID SLATS PARSLATS

SHOWER IN, VISITORS	YES	NO
SHOWER IN, EMPLOYEES	YES	NO
PERIMETER FENCE	YES	NO
CLEAN BETWEEN GROUPS	YES	NO
DISINFECT BETWEEN GROUPS	YES	NO
PQA III	YES	NO
BIRD PROOF	YES	NO
DEADS REMOVED DAILY	YES	NO
DEAD REMOVAL OFF SITE FACILITY	YES	NO
BOOT CHANGE ON ENTRY	YES	NO
BOOT HOSE	YES	NO
BOOT HOSE NOZZLE	YES	NO
BOOT BATH	YES	NO
TRANSPORT CL/SAN BEFORE	YES	NO
TRANSPORT CL/SAN AFTER	YES	NO
SEPARTE TRAILER FOR SAILES	YES	NO
TRANSPORT PERSONNEL IN BLDG	YES	NO
HOLDBACKS IN ROOM BLDG OR SITE	YES	NO

SOURCE OF FEED FARM MILL
TYPE OF FEED MEAL PELLET
MEDICATIONS

HEALTH MANAGEMENT:

HEALTH PROBLEMS AT SOURCE: NO STREP TGE SCOURS SALM RHINITIS PRRS HPS
OTHER:

HEALTH PROBLEMS IN FINISHER: NO STREP TGE SCOURS SALM RHINITIS PRRS SIV
APP HPS ASUIS OTHER
OTHER:

MEDICATIONS USED: TYPE
FEED
WATER
INJECTABLES

or building management. The fourth section asked for information about biosecurity topics. The fifth and sixth sections asked for information about the disease history of the finisher and feed information, respectively.

Data collected from the questionnaire was used to evaluate risk factors and changes in biological performance that might have been associated with high seroprevalence of *Salmonella*.

Tabulation of data collected from the questionnaire used for epidemiologic study number three

The data collected from the questionnaire was used in two different manners. First, information from the "Pig Information" section was used to determine group performance values such as average daily gain, feed efficiency, death loss, and facility turns. Then, from the "Site Information" section, the external dimensions of the finishing building(s) used by the group of pigs was used to determine the number of pounds of pork that were generated in the building for that group of pigs. Then, based on the facility efficiency for that group of pigs, a projected "pounds of pork per square foot of building per year " was calculated according to Brumm (1993) (Brumm, 1993 #962). Each of the other items of the questionnaire were then assigned an audit number based on the presumed relative risk to the other members of the category. The lower the presumed risk for the development of *Salmonella*, the lower the audit number assigned to that parameter. The presumed relative risk values for these items were assigned based on what appeared to be risks referred to in the review of the literature section of this dissertation. Table 3 contains a summary of the

management categories, their respective parameters and assigned values, and a brief definition of each parameter.

The number of weeks needed to fill the finishing facility was calculated from the information provided by the producer in the "Pig Information" section of the questionnaire.

An "Audit Score" was then calculated by adding together each of the number of weeks needed to fill the facility, "Biosecurity Information" and "Site Information"

Table 3. Explanation of the categories and parameters used in the audit form (Table 1).

Category	Parameter	Assigned Value	Definition
PIG FLOW	CONT	5	Continuous Pig Flow
	AIAO-PEN	4	All-in-all-out by pen
	AIAO-ROOM	3	All-in-all-out by room
	AIAO-BLDG	2	All-in-all-out by bldg
	AIAO-SITE	1	All-in-all-out by site
FARM TYPE	CONNECTED	3	Grower and Finisher Connected
	COMBINED	2	Grower and Finisher Combined
	FINISHER ONLY	1	No Grower Bldg, only Finisher Bldg
FEEDING SYSTEM	DRY	2	Dry feed feeders
	WET	1	Wet feed feeders
	WET/DRY	3	Dry feed feeders with nipples
BAIT STATIONS	YES	0	Bait stations used
	NO	1	No bait stations used
VENTILATION	NATURAL	0	No fans used
	POWER	1	Fans used
MANURE REMOVAL	PIT	0	Deep pit under bldg
	FLUSH	1	Manure flushed
	SCRAPE	2	Manure scraped
FLUSH WATER	FRESH	0	Fresh water flush
	RECYCLE	1	Lagoon water flush
BIOSECURITY	YES	0	Method used/present
	NO	1	Method not used/not present
DISEASE INFORMATION	YES	1	Clinical disease present
	NO	0	No clinical disease present
FEED INFORMATON	FARM	1	Feed manufactured by farm personnel
	MILL	0	Feed toll manufactured
FEED TYPE	MEAL	1	Meal fed
	PELLET	0	Pellets fed

parameters. The final audit scores, AUDITA and AUDITS, were computed by adding to or subtracting from (respectively) the "Audit Score" the number of weeks to fill the nursery.

Analysis of data collected for epidemiologic study number three

The seroprevalence for *Y. enterocolitica, T. gondii,* and *T. spiralis* antibody was determined for each group of pigs from Farms C, D, E, and F that was tested for these antibodies.

The seroprevalence of *Salmonella* was determined for each group of pigs for which serum or meat juice was tested. Based on the *Salmonella* seroprevalence of each group, a "*Salmonella* Level" of 1, 2, or 3 was assigned as previously described (Nielsen et al. 1995). Level 1 groups of pigs had a *Salmonella* seroprevalence that was less than or equal to 10%, level 2 groups of pigs had *Salmonella* seroprevalence that was greater than 10% and less than or equal to 15%. Level 3 groups of pigs had *Salmonella* seroprevalence that was greater than 15%.

The seroprevalence for each group of pigs was compared for associations with each of the various audit category parameters.

The associations between pig growth as measured by pounds of pork (liveweight) generated per square foot of building per year was compared for each of the three levels of *Salmonella* seroprevalence.

Statistical analyses were performed using commercially available statistics software as previously described on page 69.

Odds ratios were calculated as previously described (Thrusfield 1995) using the two-by-two table construction for the comparison of factors (Table 4).

Table 4. Two-by-two table used to calculate odds ratio

	DISEASE	
RISK FACTOR	**PRESENT (+)**	**ABSENT (-)**
PRESENT	**A**	**B**
ABSENT	**C**	**D**

Odds ratio (ψ) was then calculated as follows:

$$\psi = (A \times D) / (B \times C)$$

The variance (σ) of the data is estimated by using the following formula:

$$\sigma = 1/A + 1/B + 1/C + 1/D$$

The 95% confidence interval (95% C.I.) is calculated for the calculated odds ratio and its variance:

$$95\%\ C.I. = \psi\ x\ (\exp(-1.96 \times \sqrt{\sigma}),\ \psi\ x\ (\exp(1.96 \times \sqrt{\sigma})$$

Odds ratios greater than 1 and with a 95% C.I. with a lower limit greater than one were considered to be significant.

CHAPTER FOUR. RESULTS

Vaccine studies

Vaccine study one. Vaccination of pigs at one day of age followed by challenge at 35 days of age with *S. choleraesuis*

Salmonella status of sows used to produce pigs for vaccine study number one

All fecal samples collected from the 15 sows in this study were culture-negative for *Salmonella*. Each of the serum samples from all of the sows had an OD% greater than 10 and were considered to be seropositive for *Salmonella* and thus had previous exposure to *Salmonella*.

Clinical signs

The average clinical score (10.00) of pigs from the vaccinated group were significantly (p=.0001) better than the average clinical score (12.2) of pigs from the nonvaccinated group of pigs. However, analysis of the data also showed an interaction between treatment and farrowing crate which was significant (p=.0001).

There were significant ($p < .05$) differences in clinical scores among *S. choleraesuis*-challenged treatment groups following challenge. Average clinical scores for each treatment group that was challenged with *S. choleraesuis* were expressed as least square means and are summarized in Table 5.

Differences in mean daily clinical scores were significant when the two vaccine groups (Treatment Groups 1 and 2) were compared and when the vaccinated groups of pigs were compared with the nonvaccinated groups of pigs. Figure 1 contains a graphic

Table 5. Comparison of average clinical scores (least square means, post challenge) for each treatment group of vaccine study number 1 following challenge with *S. choleraesuis*. Treatment Group 1 pigs were vaccinated at one day of age. Treatment Group 2 pigs were vaccinated at 21 days of age. Treatment Group 3 pigs were not vaccinated. All three treatment groups were challenged with *S. choleraesuis* at 35 days of age.

	Treatment Group		
Parameter	**1**	**2**	**3**
Mean Clinical Score	10.9^{a}	12.5^{b}	19.5^{c}
95% C. I.	10.8 - 11.0	11.6 - 13.4	18.2 - 20.7

^{a,b,c} **Means in same row with difference superscripts are statistically significant, $p<.05$.**

representation of the mean and 95% C.I. for each treatment group's clinical score for the duration of the challenge portion of vaccine study number one. After least square means analysis, the differences between vaccinates and nonvaccinates were significant beginning on day 2 following challenge and remain significant for the duration of the study. Both groups of vaccinates had lower clinical scores than the nonvaccinates.

Rectal temperatures

Mean rectal temperatures for each treatment group are summarized in Table 6. Rectal temperatures were significantly different on day -6 relative to challenge. There were no significant differences among treatment groups in any of the mean rectal temperatures that were recorded from days -5 through day -1 relative to challenge.

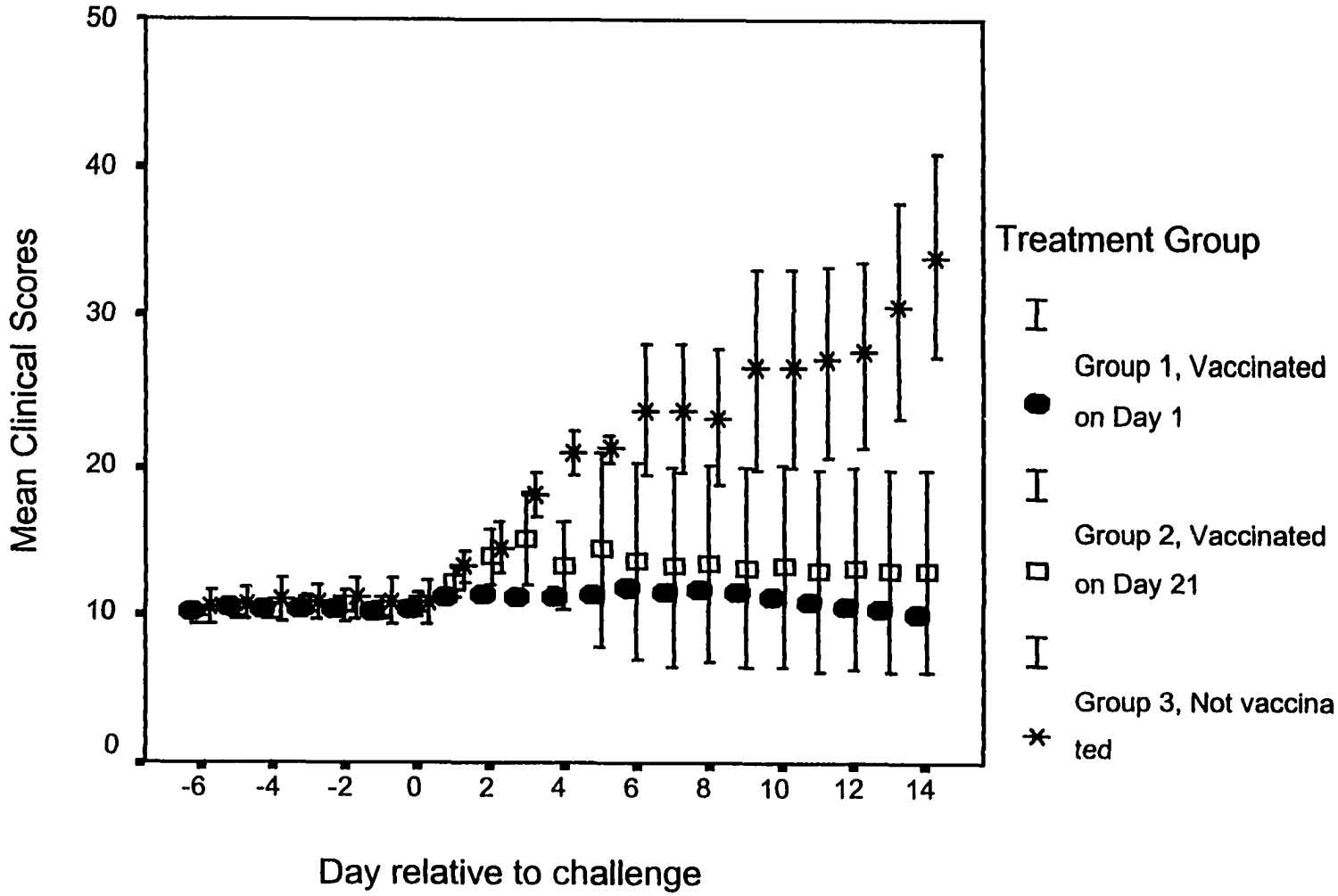

Figure 1. Comparison of mean clinical scores for the three treatment groups of vaccine study number 1.

Table 6. Average daily rectal temperatures for each treatment group for days 0 through 14. Treatment Group 1 was vaccinated at one day of age. Treatment Group 2 was vaccinated at 21 days of age. Treatment Group 3 was not vaccinated. All three groups of pigs were challenged with *S. choleraesuis* at 35 days of age.

	Treatment Group		
	1	2	3
Mean Rectal Temperature, °C	39.85[a]	40.19[b]	40.72[c]
95% C.I.	39.79 - 39.92	40.09 - 40.27	40.59 - 40.84

[a, b, c] **Means with different superscripts are significantly different ($p < .05$).**

Figure 2 contains a graphic representation of each treatment group's mean daily rectal temperature. All three treatment groups (Groups 1, 2, and 3) developed increased mean rectal temperatures on the day after challenge (Figure 2) which returned to a stable level on day -3. The cause of this increase in rectal temperature was unknown. However, there was a significant difference in mean rectal temperature after challenge when vaccinates were compared to nonvaccinates. Vaccinates (Groups 1 and 2) had lower average daily mean rectal temperatures. There were statistical differences in mean daily rectal temperatures per day beginning on day 0, immediately following challenge with χ-3246 *S. choleraesuis*. Treatment Group 1 daily mean rectal temperatures were significantly lower than Treatment Group 2 when they are compared at days 1, 7, 8, 9, and 10 relative to challenge. These two treatment groups remained the same after day 10 relative to challenge. The mean rectal temperatures of Treatment Group 3 were higher when compared to Groups 1 and 2 for days 3-14 post-challenge.

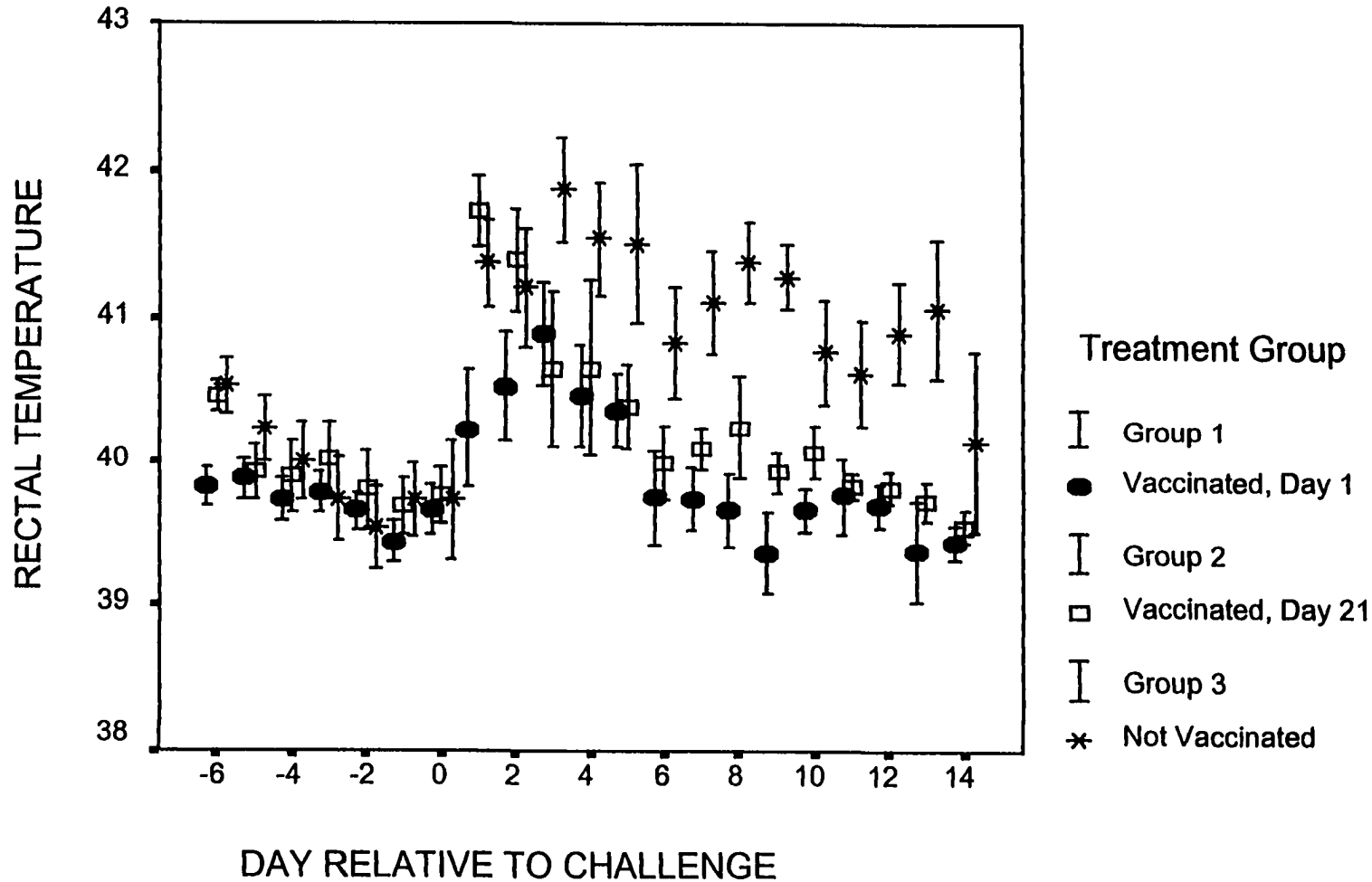

Figure 2. Comparison of mean rectal temperature (°C) for each treatment group of vaccine study number 1.

Body weight

Day 0 and day 14 mean body weight averages for pigs challenged with *S. choleraesuis* are listed in Table 7. The differences that are observed in mean pig body weight prior to challenge (beginning weight) were not significant (p = .2080) between vaccinated groups (Groups 1 and 2). Differences between vaccinates (Groups 1 and 2) and nonvaccinates (Group 3) were also not significant (p = .8814). Differences in day 14 mean body weight were significantly higher in vaccinated pigs when compared to nonvaccinated pigs (p= .0001) but the difference between the two vaccinated groups (Groups 1 and 2) was not significant (p= .9136). Nonvaccinated pigs (Group 3) lost weight during the study while vaccinates (Groups 1 and 2) gained weight. A summary of mean body weight gains is also shown in Table 7. Differences measured in mean body weight gain are significantly (p = .0001) higher in the vaccinates (Groups 1 and 2) compared to nonvaccinates (Group 3). Mean body weight gain differences were not significant among vaccinates (p=.7605).

Table 7. Mean beginning and ending body weights and weight gain in grams. Beginning weights were measured the day before challenge. Treatment Group 1 was vaccinated at one day of age. Treatment Group 2 was vaccinated at 21 days of age. Treatment Group 3 was not vaccinated. All three groups of pigs were challenged with *S. choleraesuis* at 35 days of age.

Treatment Group	Day 0 weight grams	Day 14 weight grams	Weight gain, grams
1	5,493[a]	8,785[a]	3,292[a]
2	5,425[a]	8,864[a]	3,439[a]
3	4,903[a]	3,632[b]	-1,271[b]

[a,b] Column means with different superscripts are significantly different, p < .05.

Bacteriologic examination of organs

Table 8 shows the average $\log_{10}$ *S. choleraesuis* colonies per gram of organ cultured from pigs that were challenged with *S. choleraesuis*. All pigs from the nonvaccinated group (Group 3) had significantly higher (p=.0003) organ culture levels than any of the vaccinated pigs. All organs except the lung were negative for *S. choleraesuis* in pigs from Group 1. Table 9 shows the contrasts for the mean $\log_{10}$ of organisms for the organ culture data from pigs that were challenged with *S. choleraesuis*. The number mean $\log_{10}$ of organisms per gram of organ cultured is significantly lower in the two vaccinated groups of pigs than in the nonvaccinates. Pigs that were vaccinated at one day of age (Group 1) had significantly fewer (p=.0129) organisms isolated per gram of lung cultured when compared to pigs vaccinated at 21 days of age (Group 2).

Table 8. Bacteriologic summary of organs for each treatment group. Average numbers of *Salmonella* per organ are expressed as $\log_{10}$ per gram of tissue. Treatment Group 1 was vaccinated at one day of age. Treatment Group 2 was vaccinated at 21 days of age. Treatment Group 3 was not vaccinated. All three groups were challenged with *S. choleraesuis* at 35 days of age. Necropsy was conducted when pigs died and at the end of the challenge period, day 14 post challenge

Treatment Group	Tonsil	Lung	Liver	Spleen	Mesln*	Ileum	Colon	Total
1	0.000	0.242	0.000	0.000	0.000	0.000	0.000	0.242
2	0.000	1.382	0.000	0.000	0.276	0.411	0.000	2.069
3	2.270	5.088	4.013	3.453	3.552	2.865	3.105	24.346

*** Mesenteric lymph node.**

Table 9. Contrasts of least square means of organ culture results and pneumonia scores among treatment groups from pigs challenged with *S. choleraesuis*. Organ culture data reported below is the log_{10} number of *Salmonella* colonies per gram of organ cultured. Treatment Group 1 was vaccinated at one day of age. Treatment Group 2 was vaccinated at 21 days of age. Treatment Group 3 was not vaccinated. All three groups of pigs were challenged at 35 days of age with *S. choleraesuis*.

Contrast	Tonsil	Lung	Liver	Spleen	Mesln*	Ileum	Colon	Pneumonia Score
Grp 3 vs 1,2								
Estimate	2.27	4.28	4.013	3.45	3.41	2.65	3.105	12.91
SE**, estimate	0.443	.418	.401	.509	.492	.522	.405	3.095
MS***	37.47	132.97	117.12	86.71	84.77	51.44	70.11	1212.6
F****	26.27	104.88	100.02	45.85	48.06	25.96	58.84	17.4
Pr > F*****	.0001	.0001	.0001	.0001	.0001	.0001	.0001	.0002
Grp 1 vs 2								
Estimate	0	1.14	0	0	.276	.411	0	.625
SE, estimate	.432	.431	.401	.532	.514	.545	.422	3.233
MS	.0000	8.66	0.000	0.000	.51	1.12	0	2.604
F	.0000	6.83	0.000	0.000	.29	.57	0	.04
Pr > F	1.000	.0129	1.000	1.000	.5948	.4557	1.000	.8478

* **Mesenteric lymph node**
** **Standard error**
*** **Mean square error**
**** **F-statistic**
***** **Probability of greater f**

Pneumonia

Table 9 contains the contrasts and estimates of differences among treatment groups for mean pneumonia scores. Groups 1 and 2 (vaccinates) had significantly lower mean pneumonia scores when compared to Group 3 (non-vaccinates).

Serology

None of the pigs from any of the treatment groups developed a measurable antibody response when sera were tested by the Danish mix-ELISA.

Vaccine study number two. Vaccination of pigs at 21 days of age followed by challenge with *S. typhimurium*

Salmonella status of sows

All fecal samples collected from the sows in this trial were negative for *Salmonella* by bacteriologic culture. All serum samples from the sows had an OD% greater than 10 and were considered to be positive for *Salmonella*.

Clinical signs

None of the pigs in the nonvaccinated, challenged group developed any clinical signs indicative of infection by *S. typhimurium* for any part of the study. There were no differences between the vaccinates and non-vaccinates of the *S. typhimurium*-challenged groups when clinical scores were compared. No mortality was observed for either of the two treatment groups.

Rectal temperatures

There were no significant ($p < .05$) differences in treatment group mean rectal temperatures. Treatment group mean daily rectal temperatures are represented graphically in

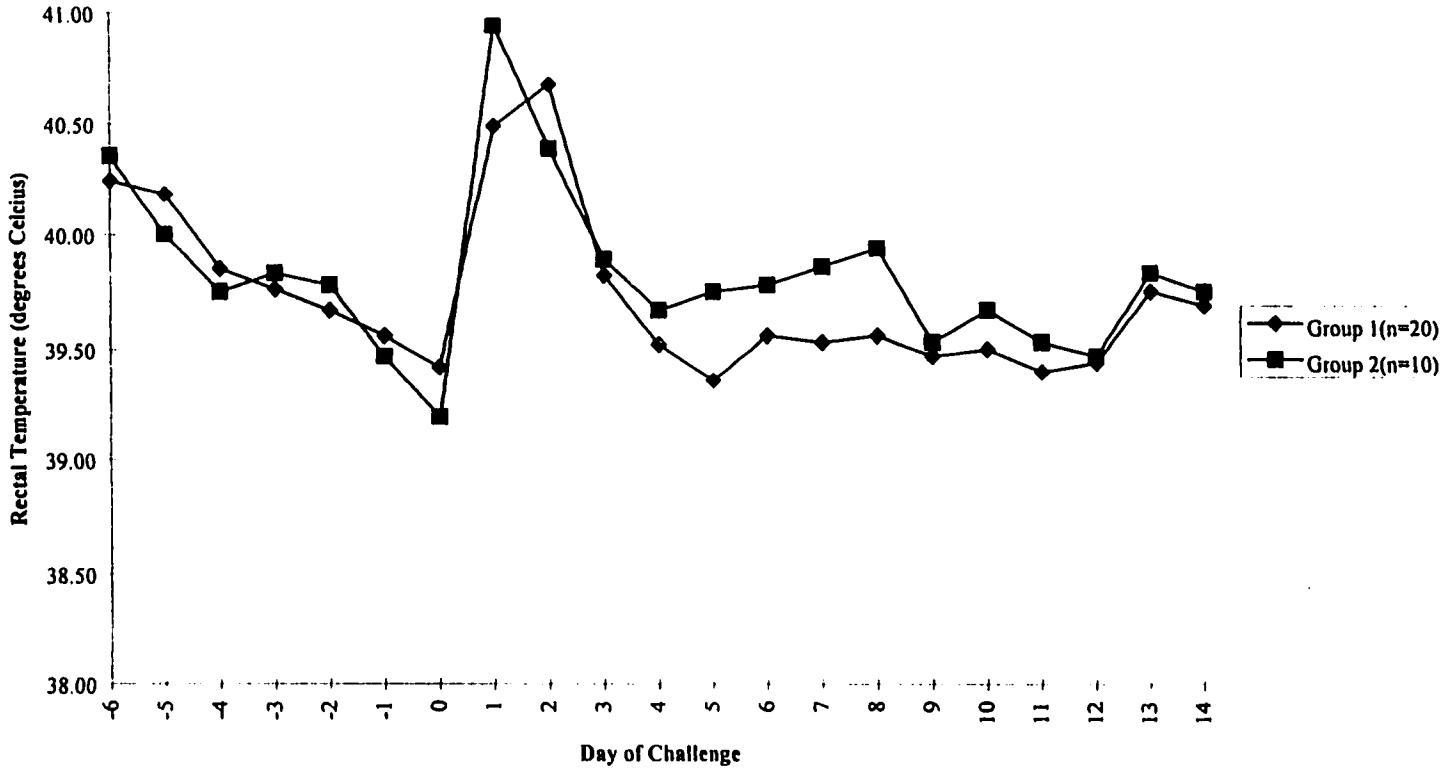

Figure 3. Average daily rectal temperatures for each treatment group of study number two. Group 1 is Treatment Group 1 and was vaccinated at 21 days of age with SC54. Group 2 is Treatment Group 2 and was not vaccinated. Both groups were challenged with S. typhimurium at 35 days of age.

Figure 3. Both groups of pigs developed increased rectal temperatures on day 1. Treatment group 1 pigs' peak rectal temperature occurred on day 1 while treatment group 2 pigs' peak rectal temperature occurred on day 2.

Pig body weight measurements

Table 10 contains a summary of the mean day 0 weight, mean day 14 weight, and mean weight gain for each treatment group. Mean day 0 body weights for vaccinates and nonvaccinates were not significantly different (p=.5243). Mean day 14 body weights for vaccinates tended to be higher than nonvaccinates but the difference was not significant (p=.0988). Mean body weight gain was significantly (p=.031) higher for vaccinates (4824 g) compared to mean body weight gain for nonvaccinates (3950 g).

Table 10. Treatment Group mean weights for *S. typhimurium*-challenged pigs in vaccine study number 2. Treatment group 1 was vaccinated at 21 days of age. Treatment group 2 was not vaccinated. Both groups were challenged with *S. typhimurium* at 35 days of age.

Treatment Group	n	Day 0 Weight, grams, ± S.E.	Day 14 Weight, grams, ± S.E.	Weight Gain, grams, ± S.E.
1	20	5,402 ± 263	10,226 ± 386	4,824 ± 199
2	10	5,130 ± 279	9,080 ± 552	3,950 ± 373
p-value		.5243	.0988	.031

Fecal shedding

All rectal swabs collected from treatment groups 1 and 2 prior to challenge were negative for *Salmonella*. A summary of the number of pigs that were shedding *S. typhimurium* is shown in Table 11. During the 14 day observation period after challenge,

Table 11. Number of pigs shedding *S. typhimurium* by day post-challenge in experiment number 2. Treatment group 1 pigs were vaccinated at 21 days of age. Treatment group 2 pigs were not vaccinated. Both treatment groups were challenged with *S. typhimurium* at 35 days of age. There were 280 pig-days for Treatment Group 1 and 140 pig-days for Treatment Group 2.

	Days Post-Challenge															
Treatment Group	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**Total**
1 (n=20)	0	2	5	3	6	0	0	0	0	1	0	2	0	1	1	21
2 (n=10)	0	4	3	6	3	0	0	0	2	1	2	3	1	1	0	26*

*** The odds likelihood of nonvaccinated pigs having more pig-days in which *S. typhimurium* is shed was 2.8 (95% C.I. 1.48 - 5.18) and was considered to be significant.**

65% (13/20) of the pigs in treatment group 1 (vaccinates) and 100% (10/10) of the pigs in treatment group 2 (non-vaccinates) were infected with and shed *S. typhimurium*. The odds ratio for nonvaccinated pigs having more pigs that shed *S. typhimurium* was 11.67 (95% CI, 0.6, 226.27) and was not considered to be significant.

Pigs from treatment group 1 shed *S. typhimurium* on 7.5% (21 positive/[20 pigs * 14 days]) of the days post-challenge and pigs in treatment group 2 shed *S. typhimurium* on 18.5% (26 positive/[10 pigs * 14 days]) of the days post-challenge (Table 11). The odds likelihood of nonvaccinated pigs having more pig-days in which *S. typhimurium* was shed was 2.8 (95% C.I. 1.48 - 5.18) and was considered to be significant ($p < .05$).

Bacteriologic examination of organs

Treatment group 1 (vaccinated) pigs had *S. typhimurium* isolated from 15% (3/20) of the animals and 2% (3/140) organs. All 3 isolations were from the tonsil. The three infected animals of treatment group 1 had *S. typhimurium* isolated from the tonsil (n=2) and the ileum (n=1). All other organs were negative. Treatment group 2 (not vaccinated) pigs had *S. typhimurium* isolated from 30% (3/10) of all animals and 4% (3/70) of the organs were infected. The differences seen among pigs and organs were compared using an odds ratio. The odds ratio of nonvaccinated pigs having organs that were positive for *S. typhimurium* was calculated to be 2.4 (95% CI: 0.3905 - 15.105) and was not considered to be significant ($p < .05$). The odds ratio of organs from nonvaccinated pigs being positive *for S. typhimurium* was calculated to be 2.04 (95% CI: 0.54 -7.66) but was not considered to be significant ($p < .05$).

Vaccine study number three. Vaccination of pigs at one day of age followed by challenge with *S. typhimurium*

***Salmonella* status of sows**

All rectal swab samples collected from sows were culture negative for the presence of *Salmonella*. All serum samples collected from sows had mix-ELISA OD% greater than 10 and were considered to be seropositive for *Salmonella*. Thus, the sows had previous exposure to *Salmonella*.

Clinical signs

Table 12 contains a summary of the treatment group mean clinical scores for the 3 days prior to challenge with *S. typhimurium*.

Table 12. A comparison of pre-challenge clinical scores by treatment group in vaccine study number 3. Treatment group 1 was pigs were not vaccinated nor were they challenged. Treatment group 2 pigs were vaccinated but not challenged. Treatment group 3 pigs were not vaccinated but were challenged with *S. typhimurium* at 35 days of age. Treatment group 4 pigs were vaccinated at 1 day of age and challenged with *S. typhimurium* at 35 days of age.

Treatment Group	n	Treatment Group	Avg. Clinical Score
Group 1	4	NN*	10.25[a]
Group 2	5	VN**	13.47[b]
Group 3	10	NC***	10.13[a]
Group 4	20	VC***	10.05[a]

[a, b,] **Clinical scores with different superscripts are significantly different, $p<.01$.**

* **Not vaccinated, not challenged**

** **Vaccinated, not challenged**

*** **Not vaccinated, challenged**

**** **Vaccinated, challenged**

Treatment group 2 pigs had a significantly ($p < .01$) higher mean clinical score when compared to treatment groups 1, 3, and 4. Table 11 contains a summary of the mean daily clinical scores for each treatment group. Throughout the post challenge period of this study, pigs in treatment groups 2, 3, and 4 had higher clinical scores than pigs that were in treatment group 1. The differences in these clinical scores were significant (Table 13).

Rectal temperatures

Table 14 contains a summary of the daily rectal temperatures for each treatment group. There were no significant ($p < .05$) differences among any of the treatment group daily rectal temperatures except days -2, 1, 4, 6, and 7.

Bacteriologic culture of organs

Table 15 contains a summary of the organ culture results from all four treatment groups. None of the observed differences were significant ($p<.05$).

Treatment groups 1 and 2 were not challenged with *S. typhimurium* and had lower organ culture results than did Treatment Groups 3 and 4. Treatment groups that were not challenged (Treatment Groups 1 and 2) became infected during the study.

Body weight changes of pigs

Body weights were measured for each pig in this study. Table 16 contains a summary of average weight in, average weight out, and average daily gain for each of the four treatment groups. There was no difference between the vaccinated pigs that were challenged and the nonvaccinated pigs that were challenged with *S. typhimurium*.

Table 13. Comparison of clinical scores for each treatment group at each day of vaccine study number 3. Treatment group 1 was pigs were not vaccinated nor were they challenged. Treatment group 2 pigs were vaccinated but not challenged. Treatment group 3 pigs were not vaccinated but were challenged with *S. typhimurium* at 35 days of age. Treatment group 4 pigs were vaccinated at 1 day of age and challenged with *S. typhimurium* at 35 days of age. All clinical score for treatment group 1 were significantly lower (p , .05) than all of the clinical scores for treatment groups 2, 3, and 4.

Days Post Challenge	Treatment Group 1	2	3	4
-3	10.25[a]	14.40[b]	10.20[a]	10.10[a]
-2	10.25[a]	13.80[b]	10.20[a]	10.05[a]
-1	10.25[a]	12.20[b]	10.00[a]	10.20[a]
0	10.00[a]	12.40[b]	10.10[a]	10.15[a]
1	10.50[a]	12.00[b]	13.10[b]	15.00[b]
2	10.00[a]	14.70[b]	12.90[b]	14.35[b]
3	10.00[a]	13.00[b]	15.20[b]	15.50[b]
4	10.25[a]	18.00[b]	17.00[b]	16.60[b]
5	10.25[a]	18.40[b]	15.90[b]	14.55[b]
6	10.00[a]	18.20[b]	14.70[b]	16.10[b]
7	12.50[a]	17.20[b]	17.20[b]	16.35[b]
8	12.25[a]	17.20[b]	14.40[b]	15.50[b]
9	12.00[a]	17.40[b]	15.20[b]	16.35[b]
10	11.00[a]	17.40[b]	15.80[b]	16.25[b]
11	10.50[a]	16.60[b]	14.30[b]	15.25[b]
12	10.50[a]	16.80[b]	14.30[b]	15.15[b]
13	10.25[a]	16.60[b]	14.50[b]	14.95[b]
14	10.25[a]	16.40[b]	14.20[b]	14.85[b]

[a,b] Values in rows with different superscripts are statistically significant ($p<.05$).

Table 14. Comparison of average rectal temperatures (°C) for each treatment group at each day of vaccine study number 3. Treatment group 1 was pigs were not vaccinated nor were they challenged. Treatment group 2 pigs were vaccinated but not challenged. Treatment group 3 pigs were not vaccinated but were challenged with *S. typhimurium* at 35 days of age. Treatment group 4 pigs were vaccinated at 1 day of age and challenged with *S. typhimurium* at 35 days of age.

	Treatment Group			
Days Post Challenge	1	2	3	4
-3	39.68[a]	39.38[a]	39.47[a]	39.54[a]
-2	40.15[a]	39.65[a]	39.32b	39.53[a]
-1	38.93[a]	39.42[a]	39.11[a]	39.36[a]
0	39.11[a]	39.11[a]	39.20[a]	38.78[a]
1	39.31[a]	39.29[a]	40.27[b]	40.52[b]
2	39.17[a]	39.72[a]	39.40[a]	39.22[a]
3	39.58[a]	39.22[a]	39.36[a]	39.33[a]
4	38.97[a]	39.69[a]	40.58[a]	39.73b
5	38.72[a]	39.68[a]	39.55[a]	39.12[a]
6	39.67[a]	39.78[a]	38.74[b]	39.25[b]
7	39.46[a]	39.79[a]	39.03[a]	38.88[a, b]
8	39.52[a]	39.25[a]	39.93[a]	39.26[a]
9	39.52[a]	39.67[a]	39.11[a]	39.29[a]
10	39.17[a]	39.38[a]	39.20[a]	39.35[a]
11	39.58[a]	40.01[a]	39.19[a]	39.49[a]
12	39.31[a]	39.58[a]	39.16[a]	39.63[a]
13	39.72[a]	39.82[a]	39.20[a]	39.79[a]
14	39.85[a]	39.52[a]	39.34[a]	39.76[a]

[a,b] Values in rows with different superscripts are statistically significant ($p<.05$).

Table 15. Comparison of bacteriological cultures from organs samples from each group of pigs for vaccine study number 3. Treatment group 1 was pigs were not vaccinated nor were they challenged. Treatment group 2 pigs were vaccinated but not challenged. Treatment group 3 pigs were not vaccinated but were challenged with *S. typhimurium* at 35 days of age. Treatment group 4 pigs were vaccinated at 1 day of age and challenged with *S. typhimurium* at 35 days of age. Average numbers of *Salmonella* per organ are expressed as log_{10} per gram of tissue.

Treatment Group	Colon	Ileum	Liver	Lung	Tonsil	MLN	Spleen	Total Organ Culture
1	.0000	.0000	.2500	.2500	.0000	.0000	.2500	0.7500
2	.6667	.2500	.2500	.5000	.2500	.2500	.0000	2.3333
3	.8000	1.000	.4000	.9000	1.000	.8000	.4000	5.3000
4	.7895	.8421	.1579	.8947	.9474	.4767	.1579	4.2632

Table 16. Comparison of performance parameters of treatment groups of pigs in vaccine study number 3. Treatment group 1 was pigs were not vaccinated nor were they challenged. Treatment group 2 pigs were vaccinated but not challenged. Treatment group 3 pigs were not vaccinated but were challenged with *S. typhimurium* at 35 days of age. Treatment group 4 pigs were vaccinated at 1 day of age and challenged with *S. typhimurium* at 35 days of age.

Treatment Group	Death Loss	Day 0 Weight (grams)	Day 14 Weight (grams)	Average Daily Gain (grams per pig per day)
Group 1	0	5,561	8,399	203
Group 2	0	4,653	7,264	186
Group 3	.1	4,857	5,599	61
Group 4	.15	5,448	6,649	78

Vaccine study number four. Effect of vaccination on culture and serology of commercial swine

Bacteriologic culture comparison of vaccinated pigs and nonvaccinated pigs

Table 17 contains a summary of the mesenteric lymph node culture results from samples collected from pigs that were vaccinated and from those pigs that were not vaccinated. Mesenteric lymph node cultures from pigs that were vaccinated had significantly ($p < .0001$) lower culture prevalence of *Salmonella* when compared to pigs that were not vaccinated.

Table 17. Comparison of mesenteric lymph node culture results between pigs that were vaccinated and pigs that were not vaccinated in vaccine study number 4.

Treatment Group	n	Culture Prevalence of *Salmonella*	s.d.	s.e.	95% C. I.
Nonvaccinated	851	24.2%	42.86	1.47	21.32 - 27.09
Vaccinated	815	11.9%*	32.54	1.14	9.79 - 14.26

*** The difference in culture prevalence was significant, $p < .0001$.**

Table 18 contains a summary of the *Salmonella* serogroups that were isolated from mesenteric lymph nodes collected from vaccinated and non-vaccinated pigs at slaughter. Vaccinated pigs had fewer isolates of *Salmonella* from serogroups B and C1. The reductions in the number of mesenteric lymph nodes containing serogroups B and C1 were significant ($p = .0209$ and $p=.0001$, respectively). Vaccinated pigs had significantly higher isolations of *Salmonella* serogroups C2 and E ($p=.0510$ and $p=.0007$, respectively).

Table 18. Summary of *Salmonella* serogroups and the number of isolates from each serogroup that were isolated from mesenteric lymph nodes collected at the time of slaughter from vaccinated and nonvaccinated pigs in vaccine study number four.

	Number of isolates for each treatment group		
Serogroup	Vaccinated Pigs	Non-Vaccinated Pigs	p-value of difference
B	68	100	.0209
C1	12	105	.0001
C2	6	1	.0510
E	11	0	.0007
Total	97	206	<.05

Table 19 contains a summary of the *Salmonella* serotypes that were isolated from vaccinated and non-vaccinated pigs at slaughter. There is a significant difference in the number of mesenteric lymph nodes obtained from vaccinated pigs that were negative for the presence of *Salmonella* when compared with the number of lymph nodes obtained from nonvaccinated pigs that were negative for the presence of *Salmonella*. The following serotypes were isolated significantly fewer times from vaccinated pigs: *S. derby, S. heidelberg, S. choleraesuis,* and *S. hartford* (p = .0001, .0032, .0001, and .0389, respectively). The following serotypes were isolated from vaccinated pigs at a significantly higher frequency than from nonvaccinated pigs: *S. anatum, S. agona, S. muenchen, and S. brandenburg* (p = .0007, .0121, .0221, and .0510, respectively). There was no significant difference in the isolation rates of *S. typhimurium, S.* 4, 12; i monophasic, *S. thompson, S. manhattan, and S. braenderup* (*p*= .4057, .0899, .6897, .9756, .0899, respectively).

Table 19. Summary of *Salmonella* serotypes that were isolated from mesenteric lymph nodes collected at slaughter from vaccinated and nonvaccinated pigs.

Serotype	Serogroup	O Antigens	Number of isolates for each treatment group		p-value of difference of number of isolates
			Vaccinated Pigs	Nonvaccinated Pigs	
None (negative)	N/A	N/A	718	645	.0001
S. derby	B	1, 4, 5, 12	24	60	.0001
S. typhimurium	B	1, 4, 5, 12	32	27	.4057
S. 4,12; i monophasic	B	4, 12	0	3	.0899
S. heidelberg	B	4, 12	0	9	.0032
S. choleraesuis	C1	6, 7	9	92	.0001
S. anatum	E	3, 10	11	0	.0007
S. agona	B	4, 12	6	0	.0121
S. hartford	C1	6, 7	1	7	.0389
S. thompson	C1	6, 7	2	3	.6897
S. manhattan	C2	6, 8	1	1	.9756
S. braenderup	C1	6, 7	0	3	.0899
S. muenchen	C2	6, 8	5	0	.0221
S. brandenberg	B	4, 12	6	1	.0510

Serologic comparison of vaccinated and nonvaccinated pigs

A problem occurred during processing of the meat juice samples. All meat juice samples were collected in sterile plastic bags and frozen after arriving at the laboratory facilities. When the meat juice samples were removed from the freezer and thawed, almost all of the samples contained particulates and many of the samples were cloudy. Attempts were made to centrifuge each sample in order to remove some of these particulates.

The mean OD% for each of the two treatment groups was determined. Table 20 contains a summary of the mix-ELISA results from meat juice collected from vaccinated and nonvaccinated pigs. Vaccinated pigs had a significantly lower mean OD% when compared to nonvaccinated pigs.

Table 20. Comparison of mean OD% for pigs that were in buildings that were vaccinated compared to pigs that were in buildings that were not vaccinated in vaccine study number 4.

Treatment Group	n	Mean OD%	s.d.	s.e.	95% C.I.
Nonvaccinated	822	52.2	38.2	1.33	49.6 - 54.8
Vaccinated	806	43.6	28.0	.99	41.7 - 45.5

The seroprevalence for culture-negative and culture-positive pigs were determined and compared. The difference in seroprevalence between these two groups was statistically significant ($p < .05$). Both of the groups had a seroprevalence of over 93%. Table 21a contains a summary of a comparison of the pigs from which *Salmonella* had been isolated (culture status = negative) and those pigs from which no *Salmonella* had been isolated. Using an mix-ELISA OD% cut-off of 40, 53.54% of the 297 culture-positive pigs had an

OD% greater than 40 while 46.7% of the 1331 culture-negative pigs had an OD% greater than 40. This difference was significant (p = .0423).

Table 21b contains a comparison of the percent of vaccinated pigs that were seropositive with the percent of nonvaccinated pigs that were seropositive. The difference in the percent positive was significant (p < .05).

Table 21a. Comparison of the seroprevalence (OD% cut-off > 40) of samples between culture status of all samples collected from pigs that were from buildings that were vaccinated and from buildings that were nonvaccinated in vaccine study number 4.

Culture Status	n	Seroprevalence	s.d.	s.e.	95% C.I.
Negative	1331	46.70%	49.91	1.37	44.05 - 49.42
Positive	297	53.54%*	49.96	2.90	47.83 - 59.24

*** Difference in seroprevalence was significant, p = .0423.**

Table 21b. Comparison of the percent of the vaccinated pigs that were seropositive (OD% cutoff >40) with the percentage of the nonvaccinated pigs that were seropositive in vaccine study number 4.

Treatment Group	n	% Positive	s.d.	s.e.	95% C.I.
Nonvaccinated	822	51.46	50.01	1.74	48.04 - 54.88
Vaccinated	806	44.42	49.72	1.75	40.98 - 47.85

Table 22 contains a comparison of the mean OD% between pigs that culture-positive versus those pigs that were culture-negative. Pigs that had mesenteric lymph nodes that were negative for *Salmonella* had a significantly lower mean OD% when compared to pigs that had mesenteric lymph nodes that were positive for *Salmonella*.

Table 22. Comparison of the mean OD% between pigs that were culture-positive and those pigs that were culture-negative in vaccine study number 4.

Culture Status	n	Mean OD%	s.d.	s.e.	95% Confidence Interval
Negative	1331	46.28	32.41	0.89	44.54 - 48.02
Positive	297	55.38	38.74	2.24	50.96 - 59.81

Each group of pigs was assigned to a *Salmonella* Level based on the seroprevalence of *Salmonella* antibodies as measured by the mix-ELISA. Table 23 contains a summary of all of the groups of pigs, classified by vaccination status and Danish *Salmonella* level. The mix- ELISA cut-off used to determine seropositive was greater than 40. There were no differences between vaccinates and nonvaccinates when any of the three *Salmonella* levels were compared.

Table 24 contains a summary of all of the groups of pigs, classified by vaccination status and Danish *Salmonella* level. The mix-ELISA cut-off used to determine seropositive was greater than 100. Using the higher cut-off, groups of pigs that were vaccinated had a

Table 23. Classification of all groups of pigs according to their vaccination status and their *Salmonella* level in vaccine study four. A mix-ELISA OD% of 40 was used as a cut-off for a seropositive sample of meat juice. *Salmonella* level of 1 had less than or equal to 10% of the group of pigs that tested seropositive for *Salmonella* antibodies. *Salmonella* level of 2 had more than 10% but less than or equal to 15% of the group of pigs that tested seropositive for *Salmonella* antibodies. *Salmonella* level of 3 had more than 15% of the group of pigs that tested seropositive for *Salmonella* antibodies.

	Salmonella Level		
Treatment	**1**	**2**	**3**
Not Vaccinated	5	2	21
Vaccinated	2	3	23

Table 24. Classification of all groups of pigs according to their vaccination status and their *Salmonella* level in vaccine study number 4. A mix-ELISA OD% of 100 was used as a cut-off for a seropositive sample of meat juice. *Salmonella* level of 1 had less than or equal to 10% of the group of pigs that tested seropositive for *Salmonella* antibodies. *Salmonella* level of 2 had more than 10% but less than or equal to 15% of the group of pigs that tested seropositive for *Salmonella* antibodies. *Salmonella* level of 3 had more than 15% of the group of pigs that tested seropositive for *Salmonella* antibodies.

	Salmonella Level		
Treatment	**1**	**2**	**3**
Not Vaccinated	19	2	7
Vaccinated	24	3	1

higher occurrence of level 1 groups and a lower occurrence of level 3 groups when compared to the nonvaccinated groups of pigs.

Vaccine study number five. Serologic response of pigs vaccinated at 21 days of age.

Thirty pigs from each of the treatment groups were bled 90 days following the administration of vaccine. The average OD% for the vaccinated group of pigs was than 10 . The average OD% for the nonvaccinated group of pigs was also less than 10.

Epidemiologic studies

Epidemiologic study number one. Serologic and bacteriologic studies of blood and fecal samples collected from three farms

Culture results from all farms sampled

There were 1,882 pen fecal samples collected from 47 groups of pigs belonging to the 3 different farms. The sample prevalence was 2.66% (50/112). Eighteen groups of finishing pigs were positive for *Salmonella* for a group prevalence of 38.3%. Summaries of samples collected from each farm are outlined in Tables 25-27.

Table 25. Summary of *Salmonella* isolates from Farm A environmental pen fecal samples.

Month	Building	No. Pen Fecal Samples	No. positive	Percent Positive	Serotypes Isolated
Dec-94	4A	25	0	0	0
Jan-95	6C	90	0	0	0
Feb-95	6A	75	0	0	0
Mar-95	6A	75	0	0	0
Apr-95	none	0	0	0	0
May-95	4A	60	3	5	*S. enteritidis*
Jun-95	6C	60	0	0	0
Jul-95	6C	60	0	0	0
Aug-95	6A	30	2	7	*S. derby* *S. thomasville*
Sep-95	4A	60	0	0	0
Oct-95	6A	30	0	0	0
Nov-95	none	0	0	0	0
Dec-95	6C	25	0	0	0
Total		590	5	1	

Table 26. Summary of *Salmonella* isolates from Farm B environmental pen fecal samples.

Month	Building	No. Pen Fecal Samples	No. Positive	Percent Positive	Serotypes Isolated
Jan-95	Confinement	40	4	10	*S. derby*
Feb-95	Confinement	100	4	4	*S. derby*
Mar-95	Confinement	20	0	0	0
Apr-95	Confinement	60	0	0	0
May-95	Confinement	60	0	0	0
Jun-95	Cargill	60	0	0	0
Jul-95	Confinement	60	0	0	0
Aug-95	Cargill	30	0	0	0
Sept-95	Confinement	60	0	0	0
Oct-95	Confinement	30	0	0	0
Nov-95	Confinement	30	0	0	0
Dec-95	Confinement	30	3	10	*S. choleraesuis*
Total		580	11	1.9	

Table 27. Summary of *Salmonella* isolates from Farm C environmental pen fecal samples.

Month	Building	No. Positive	No. Pen Fecal Samples	Percent Positive	Serotypes Isolated
Dec-94	U	4	28	14	*S. heidelberg*
Jun-95	H	5	30	17	*S. anatum, S. typhimurium, (Copenhagen) (2), S. derby, S. heidelberg*
Jun-95	I	5	30	17	*S. heidelberg, S. anatum (4)*
Jun-95	M	0	30	0	
Jun-95	V	0	30	0	
Jun-95	W	1	30	3	*S. choleraesuis*
Aug-95	Q	2	30	7	*S. derby S. heidelberg*
Aug-95	O	0	30	0	
Sep-95	O	0	30	0	
Sep-95	P	1	30	3	*S. heidelberg*
Sep-95	S	0	30	0	
Sep-95	J	2	28	7	*S. derby, S. infantis*
Oct-95	M	2	30	7	*S. derby (4)*
Oct-95	N	0	30	0	
Oct-95	R	0	30	0	
Oct-95	U	4	30	13	*S. derby (2) S. typhimurium, (Copenhagen) (2)*
Nov-95	H	0	30	0	
Nov-95	I	0	30	0	
Nov-95	V	1	30	3	*S. derby*
Dec-95	E	3	30	10	*S. heidelberg*
Dec-95	W	3	30	10	*S. derby (2) S. heidelberg*
Dec-95	L	1	30	3	*S. derby*
Dec-95	I	0	30	0	

Eight different serotypes of *Salmonella* were isolated among the 50 culture-positive pen fecal samples. The frequency of the eight different serotypes ranged from 2-40% (Table 28). The most frequently isolated serotypes were *S. derby* and *S. heidelberg*.

The Kaufmann-White O antigen scheme of each of these serotypes is shown in Table 29 (Guthrie 1992).

Table 28. Serotypes isolated from each of the pen fecal samples.

Serotypes Isolated	No. of serotypes isolated	Percent of all serotypes isolated
S. derby	20	40
S. heidelberg	12	24
S. anatum	5	10
S. typhimurium (including Copenhagen)	4	8
S. choleraesuis	4	8
S. enteritidis	3	6
S. infantis	1	2
S. thomasville	1	2
Total	50	100

Table 29. Kaufmann-White O antigen content of *Salmonella* serotypes isolated from pen fecal samples.

Serotype	O Group	O Antigens
S. derby	B	1, 4, 5, 12
S. heidelberg	B	[1], 4, [5], 12
S. anatum	E1	3, 10
S. typhimurium (including Copenhagen)	B	1, 4, 5, 12
S. choleraesuis var. Kunzendorf	C1	6, 7
S. enteritidis	D1	1, 9, 12
S. infantis	C1	6, 7, [14]
S. thomasville	E3	(3), (15), 34

( ) = antigen incomplete [ ] = antigen may be present or absent

Culture results from Farm A

Farm A had no previous history of *Salmonella* infection in its pigs. Pen feces were collected from all available pens in each building. On two occasions, *Salmonella* were isolated from pen fecal samples collected from two different buildings (Table 25). These isolations occurred during the Spring (April-June) and during the Summer (July-September). *S. enteritidis, S. derby*, and *S. thomasville* were the three serotypes isolated from Farm A environmental pen fecal samples. The sample culture prevalence for Farm A was 0.9%. The group prevalence was 18%.

Culture results from Farm B

Farm B had a previous history of *Salmonella* infection in its pigs. *Salmonella choleraesuis* had been identified as the etiologic agent involved in those infections. Pen feces were collected from all available pens in each building. Table 26 contains a summary of the culture results from pen fecal samples. *Salmonella choleraesuis* was isolated only in the last month of the testing period. *Salmonella derby* was isolated from a total of 8 fecal samples from samples collected in January and February of 1995. *Salmonella choleraesuis* was isolated from the environmental pen fecal samples that were collected in December, 1995. The sample culture prevalence for Farm B was 1.9% and the group culture prevalence for Farm B was 25%.

Culture results from Farm C

Twenty-five different groups of pigs from fifteen different buildings were sampled (Table 27). Randomly selected pens from each building were sampled. *Salmonella* was

isolated from 13 groups of pigs. The different serotypes of *Salmonella* and their frequency of isolation are described in Table 30.

Salmonella heidelberg and *S. derby* were isolated more frequently than any of the other 4 serotypes isolated from these buildings. The other serotypes that were isolated were *S. anatum, S. typhimurium* (including copenhagen), *S. choleraesuis*, and *S. infantis*. The sample culture prevalence for Farm C was 4.96%. The group culture prevalence was 56.5%.

Table 30. Serotypes of *Salmonella* isolated from Farm C.

Serotypes Isolated	No. Isolated	Percent
S. heidelberg	12	34
S. derby	11	32
S. anatum	5	15
S. typhimurium (including copenhagen)	4	12
S. choleraesuis	1	3
S. infantis	1	3
Total	34	100

Analysis of culture results

Culture prevalence of *Salmonella* was compared for the following parameters: use of an isolated nursery, season of collection and farm. There were not enough data points to analyze prevalence differences among groups of pigs tested from isolated nurseries or from different seasons of the year. However, it appeared that prevalence differences could be associated with season and the use of an isolated nursery. Table 31 summarizes one-way analysis of variance of the culture prevalence of *Salmonella* for each farm. When environmental fecal samples from the three farms were compared (Table 31), Farm C had a significantly ($p < .05$) higher culture prevalence of *Salmonella* than Farms A and B.

Table 31. Comparison of culture prevalence of *Salmonella* from environmental fecal samples collected from each farm. Samples were collected from Farm A and Farm B for one year. Samples were collected from Farm C for 6 months of a year. Farm C has a significantly ($p < .05$) higher prevalence of *Salmonella* when compared to Farms A and B.

Farm	n	Mean Percent Positive	S.D.	S.E.	95% C.I.
A	590	.0085	.0917	.0038	.0011 - .0159
B	580	.0190	.1365	.0057	.0078 - .0301
C	712	.0478	.2134	.0080	.0321 - .0635
Total	1882	.0266	.1609	.0037	.0193 - .0338

Serology results from all farms sampled

There were 1476 serum samples collected from 47 groups of finishing pigs from farms A, B, and C at the same time that environmental fecal samples were collected. There were 479 serum samples that were positive for *Salmonella* antibodies when tested by the Danish mix-ELISA. There were 23 groups of pigs that were seropositive (>10% of group seroprevalence using OD% > 10). The sample serologic prevalence was 32.5% (479/1476). The group serologic prevalence was prevalence was 48.94 % (23/47). Tables 32-35 each contain a monthly summary of all of culture and serology results for Farms A, B, and C, respectively. Results for bacteriologic and serologic testing for each month of testing from Farms A and B represented a group of pigs (Table 35, 36). Table 37 contains a summary of all of the groups of pigs that were tested from Farm C. Environmental fecal samples were collected from Farm C only during the period of June, 1995, through December, 1995. Samples of blood and environmental fecal samples were not collected during the month of July from Farm C.

Serology results from Farm A

A summary of the results from mix-ELISA testing of serum collected from pigs from Farm A is contained in Table 32. When a mix-ELISA OD% > 10 was used as a cut-off for seropositive results, there were 6 groups of pigs that had over 10% seroprevalence of *Salmonella* antibodies by the mix-ELISA. The month with highest seroprevalence occurred in September. The lowest seroprevalence occurred in the months of May and June. Two of the three months in which *Salmonella* was isolated (Table 35) had over 10% seroprevalence and were considered to be seropositive. The average monthly seroprevalence for Farm A was 15.6% using mix-ELISA OD% cut-off greater than 10 for seropositive. Each group of pigs had less than 10% seropositive when using mix-ELISA OD% cut-off greater than 40 for seropositive. When the higher OD% cut-off was used to classify the groups of pigs from this farm, all groups of pigs were classified as *Salmonella* level 1.

Serology results from Farm B

A summary of the results from mix-ELISA testing of serum collected from pigs from Farm B is contained in Table 33. There were 6 groups of pigs that had over 10% seroprevalence of *Salmonella* antibodies by the mix-ELISA using an OD% cut-off of greater than 10 for seropositive. The highest seroprevalence occurred in August and October. The lowest seroprevalence occurred in the months of January, February, September, and November. None of the three months in which *Salmonella* was isolated (Table 36) were considered to be seropositive. The average monthly seroprevalence for Farm B was 11.5% when using an OD% cut-off of 10. All groups of pigs that were tested from Farm B were classified as *Salmonella* level 1, using a seropositive cut-off value of OD% > 40.

Table 32. Summary of mix-ELISA results from monthly serum samples collected from pigs from Farm A. The *Salmonella* Level is determined by the percent of the samples that are positive, using an OD% cut-off of greater than 40. A level 1 farm has less than or equal to 10% positive. A level 2 has over 10% and less than or equal to 15% of the samples positive. A level 3 farm has over 15% of the samples positive.

Month	No. Serum Samples	No. Positive (OD%>10)	% Positive (OD%>10)	No. Positive (OD%>40)	% Positive (OD%>40)	*Salmonella* Level
Jan	30	2	6.7	1	6.7	1
Feb	30	5	17.7	0	0.0	1
Mar	30	7	23.3	2	6.7	1
Apr	None	None	None	N/A	None	N/A
May	39	2	5.1	0	0.0	1
Jun	30	0	0.0	0	0.0	1
Jul	34	3	8.8	0	0.0	1
Aug	29	3	10.3	0	0.0	1
Sep	57	21	38.6	4	7.0	1
Oct	30	4	13.3	0	0.0	1
Nov	None	None	None	N/A	None	N/A
Dec	61	10	16.4	3	4.9	1
Total	370	58	15.6	10	2.7	

Table 33. Summary of mix-ELISA results from monthly serum samples collected from pigs from Farm B. The *Salmonella* Level is determined by the percent of the samples that are positive, using an OD% cut-off of greater than 40. A level 1 farm has less than or equal to 10% positive. A level 2 has over 10% and less than or equal to 15% of the samples positive. A level 3 farm has over 15% of the samples positive.

Month	No. Samples	No. Positive (OD%>10)	% Positive (OD%>10)	No. Positive (OD%>40)	% Positive (OD%>40)	Salmonella Level
Jan	30	1	3	0	0	1
Feb	30	1	3	0	0	1
Mar	30	4	13	1	3	1
Apr	29	3	10	0	0	1
May	30	3	10	0	0	1
Jun	30	6	20	0	0	1
Jul	30	5	17	0	0	1
Aug	29	8	28	0	0	1
Sep	30	0	0	0	0	1
Oct	30	8	27	0	0	1
Nov	30	0	0	0	0	1
Dec	30	2	7	0	0	1
Total	358	41	11.5	1	0.3	

Table 34. Summary of mix-ELISA results from monthly serum samples collected from pigs from Farm C. The *Salmonella* Level is determined by the percent of the samples that are positive, using an OD% cut-off of greater than 40. A level 1 farm has less than or equal to 10% positive. A level 2 has over 10% and less than or equal to 15% of the samples positive. A level 3 farm has over 15% of the samples positive.

Month	No. Samples	No. Positive (OD%>10)	% Positive (OD%>10)	No. Positive (OD%>40)	% Positive (OD%>40)	Salmonella Level
Dec	58	11	19	3	5	1
Jan	150	19	13	6	4	1
Feb	150	16	11	7	5	1
Mar	115	15	13	6	5	1
Apr	118	15	13	0	0	1
May	121	53	44	27	22	3
Jun	151	36	24	15	10	1
Jul	None	None	None	None	None	None
Aug	60	43	72	28	45	3
Sep	118	103	87	59	50	3
Oct	126	91	72	41	33	3
Nov	117	52	44	25	21	3
Dec	117	52	44	44	38	3
Total	1401	506	36.1	261	18.6	

Serology results from Farm C

A summary of the results from mix-ELISA testing of serum collected from pigs from Farm C is contained ins Table 34 and 37. Twenty-four of the 46 groups of pigs were seropositive (cut-off = OD%>10). The highest seroprevalence occurred in August, September, and October. The lowest seroprevalence occurred in the months of January, February, and March. The seroprevalence of *Salmonella* antibodies was highest in every month in which *Salmonella* was isolated from environmental pen fecal samples. The average monthly sample seroprevalence for Farm C was 18% (cut-off = OD%>40). There were 22 groups of pigs that were classified as *Salmonella* level 1, 5 that were classified as *Salmonella* level 2, and 19 that were *Salmonella* level 3.

Analysis of culture and serology results

All culture and serologic results are summarized for each farm in Tables 35-37. Farms A and B have lower culture and serologic prevalence of *Salmonella* than Farm C. The lower culture and serologic prevalence of Farms A and B are also associated with a *Salmonella* Level of 1. Farm C's higher prevalence of *Salmonella* is associated with more groups of pigs being designated as *Salmonella* Level 3.

A summary of analysis of variance of the seroprevalence of *Salmonella* for all groups of pigs from all farms are in Table 38. Culture-positive groups of pigs tended to have higher seroprevalence of *Salmonella* antibody than did culture-negative groups of pigs. The observed difference was not significant ($p = .0872$).

The seroprevalence for culture-positive and culture-negative groups of pigs is summarized in Table 39.

Table 35. Summary of all environmental fecal samples and serum samples collected from Farm A. The *Salmonella* Level is determined by the percent of the samples that are positive, using an OD% cut-off of greater than 40. A level 1 farm has less than or equal to 10% positive. A level 2 has over 10% and less than or equal to 15% of the samples positive. A level 3 farm has over 15% of the samples positive.

		Environmental Fecal Samples				Serum Samples			
Month	Site	No. Collected	No. Positive	Percent Positive	Serotypes Isolated	No Collected	No. Positive (OD%>40)	Percent Positive	*Salmonella* Level
Dec-94	4A	25	0	0		36	3	8	1
Jan-95	6C	90	0	0		30	1	7	1
Feb-95	6A	75	0	0		30	0	0	1
Mar-95	6A	75	0	0		30	2	7	1
Apr-95	none					none			
May-95	4A	60	3	5	*S. enteritidis*	39	0	0	1
Jun-95	6C	60	0	0		30	0	0	1
Jul-95	6C	60	0	0		34	0	0	1
Aug-95	6A	30	2	7	*S. derby* *S. thomasville*	29	0	0	1
Sep-95	4A	60	0	0		57	4	7	1
Oct-95	6A	30	0	0		30	0	0	1
Nov-95	none					none			
Dec-95	6C	25	0	0		25	0	0	1

Table 36. Summary of all environmental fecal samples and serum samples collected from Farm B. The *Salmonella* Level is determined by the percent of the samples that are positive, using an OD% cut-off of greater than 40. A level 1 farm has less than or equal to 10% positive. A level 2 has over 10% and less than or equal to 15% of the samples positive. A level 3 farm has over 15% of the samples positive. Site C was a confinement building. Site CG was a Cargill finishing building.

		Environmental Fecal Samples				Serum Samples			
Month	Site	No. Collected	No. Positive	Percent Positive	Serotypes Isolated	No Collected	No. Positive (OD%>40)	Percent Positive	*Salmonella* Level
Jan-95	C	40	4	10	*S. derby*	30	0	0	1
Feb-95	C	100	4	4	*S. derby*	30	0	0	1
Mar-95	C	20	0	0		30	1	3	1
Apr-95	C	60	0	0		29	0	0	1
May-95	C	60	0	0		30	0	0	1
Jun-95	CG	30	0	0		30	0	0	1
Jul-95	C	60	0	0		30	0	0	1
Aug-95	CG	30	0	0		29	0	0	1
Sep-95	C	60	0	0		30	0	0	1
Oct-95	C	30	0	0		30	0	0	1
Nov-95	C	30	0	0		30	0	0	1
Dec-95	C	30	3	10	*S. choleraesuis*	30	0	0	1

Table 37. Summary of all environmental fecal samples and serum samples collected from Farm C. The *Salmonella* Level is determined by the percent of the samples that are positive, using an OD% cut-off of greater than 40. A level 1 farm has less than or equal to 10% positive. A level 2 has over 10% and less than or equal to 15% of the samples positive. A level 3 farm has over 15% of the samples positive.

		Environmental Fecal Samples				Serum Samples			
Month	Site	No. Collected	No. Positive	Percent Positive	Serotypes Isolated	No Collected	No. Positive (OD%>40)	Percent Positive	*Salmonella* Level
Dec-94	U	28	4	14	*S. heidelberg*	58	3	5	1
Jan-95	H	0	0	0		30	0	0	1
Jan-95	I	0	0	0		30	5	17	3
Jan-95	M	0	0	0		30	0	0	1
Jan-95	N	0	0	0		30	1	3	1
Jan-95	V	0	0	0		30	0	0	1
Feb-95	E	0	0	0		30	0	0	1
Feb-95	F	0	0	0		30	2	7	1
Feb-95	L	0	0	0		30	0	0	1
Feb-95	V	0	0	0		30	0	0	1
Feb-95	W	0	0	0		30	5	15	2
Mar-95	E	0	0	0		28	3	11	2
Mar-95	L	0	0	0		29	0	0	1
Mar-95	O	0	0	0		29	3	10	2
Mar-95	Q	0	0	0		30	0	0	1
Apr-95	J	0	0	0		29	0	0	1
Apr-95	K	0	0	0		30	0	0	1
Apr-95	O	0	0	0		30	0	0	1
Apr-95	S	0	0	0		29	0	0	1
May-95	M	0	0	0		30	12	40	3
May-95	N	0	0	0		30	15	50	3
May-95	R	0	0	0		29	0	0	1
May-95	U	0	0	0		32	0	0	1

Table 37. (continued)

		Environmental Fecal Samples				Serum Samples			
Month	Site	No. Collected	No. Positive	Percent Positive	Serotypes Isolated	No Collected	No. Positive (OD%>40)	Percent Positive	*Salmonella* Level
Jun-95	H	30	5	17	*S. anatum* *S. typhimurium copenhagen (2)* *S. derby* *S. heidelberg*	30	2	7	1
Jun-95	I	30	5	17	*S. heidelberg* *S. anatum (4)*	30	0	0.0	1
Jun-95	M	30	0	0		31	2	6	1
Jun-95	V	30	0	0		30	1	3	1
Jun-95	W	30	1	3	*S. choleraesuis*	30	10	33	3
Aug-95	O	30	0	0		30	19	63	3
Aug-95	Q	30	2	7	*S. derby* *S. heidelberg*	30	9	30	3
Sep-95	J	28	2	7	*S. derby* *S. infantis*	30	16	53	3
Sep-95	O	30	0	0		30	14	47	3
Sep-95	P	30	1	3	*S. heidelberg*	30	17	57	3
Sep-95	S	30	0	0		28	12	43	3
Oct-95	M	30	2	7	*S. derby*	30	4	13	2
Oct-95	N	30	0	0		30	10	33	3
Oct-95	R	30	0	0		28	14	50	3
Oct-95	U	30	4	13	*S. derby (2)* *S. typhimurium copenhagen (2)*	38	13	34	3

Table 37. (continued)

		Environmental Fecal Samples				Serum Samples			
Month	**Site**	**No. Collected**	**No. Positive**	**Percent Positive**	**Serotypes Isolated**	**No Collected**	**No. Positive (OD%>40)**	**Percent Positive**	***Salmonella* Level**
Nov-95	H	30	0	0		31	6	32	3
Nov-95	I	30	0	0		30	1	13	2
Nov-95	V	30	1	3	*S. derby*	30	8	60	3
Nov-95	W	26	0	0		26	10	77	3
Dec-95	E	30	3	10	*S. heidelberg*	30	16	80	3
Dec-95	I	30	0	0		29	0	0	1
Dec-95	L	30	1	3	*S. derby*	30	11	80	3
Dec-95	W	30	3	10	*S. derby (2)* *S. heidelberg*	28	17	86	3
Total		**712**	**34**	**5**		**1402**	**258**	**18**	

Table 38. One way ANOVA of culture status and serology (OD%>10) results for each group of pigs that was tested during this study. Groups of pigs that were culture positive were compared with groups of pigs that were culture negative with regard to the percent of seropositive samples per group.

Source	df	Sum of Squares	Mean Square	F Ratio	F Probability
Between Groups	1	3113	3113	3.1	.0872
Within Groups	45	45824	1018		
Total	46	48938			

Table 39. Summary statistics of the seroprevalence of culture-positive and culture-negative groups of pigs using a mix-ELISA cut-off of OD%>10.

Group	Count	Seroprevalence	s.d	s.e	95% CI
Culture Positive	18	45%	35	8.2	27 - 62
Culture Negative	29	28%	30	5.6	16 - 39
Total	47	34%	33	4.8	25 - 44

A summary of the mean OD% from pigs that were culture positive was compared to pigs that were culture-negative (Table 40). Culture-positive pigs have a higher antibody response to *Salmonella* when measured with the mix-ELISA. The pigs from groups that were culture-positive had a mean OD% of 24. Groups of pigs that were culture negative had a mean OD% of 13. The two means were significantly different ($p = .0001$).

Table 41 contains a summary of the groups of pigs from which blood and environmental fecal samples were collected. Each group of pigs was assigned to a *Salmonella* level based on the seroprevalence of that group of pigs. Level 1 groups of pigs have a higher percentage (72%) of low (<= 1%) culture-positive environmental fecal samples

Table 40. Summary of comparison of average percent of seropositive samples for groups of pigs that were culture-positive (positive) for *Salmonella* and groups of pigs that were culture-negative (negative) for *Salmonella*.

Group	n	Mean	s.d.	s.e.	95% C.I.
Negative	854	13	23	0.8	12 - 15
Positive	622	24	31	1.2	19 - 24
Total	1476	17	27	0.7	15 -18

Table 41. Distribution of culture-positive groups of pigs based on *Salmonella* level. *Salmonella* level was determined by the percent of seropositive (OD%>40) samples per group of pigs. *Salmonella* level 1 groups have 10% or fewer seropositive samples per group. *Salmonella* level 2 groups of pigs have over 10% and less than or equal to 15% seropositive samples per group of pigs. *Salmonella* level 3 groups of pigs have over 15% seropositive samples per group of pigs.

	Number of Groups of Pigs at each *Salmonella* Level		
Culture Prevalence	Level 1	Level 2	Level 3
<= 1%	21	1	7
> 1% and <=5%	2	0	4
>5%	6	1	5
Total	29	2	16

when compared to Level 3 groups of pigs (43.8%). Also, Level 3 groups of pigs had a higher percentage (31.3%) of high (>5%) culture prevalence groups of pigs compared to Level 1 groups of pigs (20.7%).

Epidemiologic study number two. Serologic and bacteriologic studies of meat juice and mesenteric lymph nodes collected from groups of pigs at slaughter from four farms

Introduction

Mesenteric lymph node and muscle samples from Farms A, B and C were collected only during the period of June-July, 1995. On two occasions, the slaughter plant in which

samples from A and B would have been collected were shut down due to mechanical problems. The owner of pigs from Farm B decided quit sending pigs to slaughter at this plant in November and December.

Bacteriologic examination of mesenteric lymph nodes

Tables 42-44 contain summaries of the bacteriologic examination results for the mesenteric lymph nodes that were collected from slaughter from Farms A, B, and C. Mesenteric lymph nodes were collected from pigs from Farm A during four months. Collections during the month of October were not made because of problems within the slaughter facility that prevented the killing of hogs on the day that the samples were scheduled to be collected. The culture prevalence of these samples was 9% with three difference serotypes isolated: *S. enteritidis, S. anatum,* and *S. bredeney. Salmonella bredeney* was not demonstrated to be present in any of the environmental fecal samples collected from Farm A during the course of the year.

Mesenteric lymph nodes were collected from Farm B for 4 months. Samples were to be collected in October, but were not available due to the previously-mentioned slaughter plant shut down that also prevented the collection of samples for Farm A. Farm B had an overall *Salmonella* culture prevalence of 7%. There were 3 serotypes demonstrated to be present in the mesenteric lymph nodes of pigs from Farm B: *S. anatum, S. typhimurium copenhagen, and S. agona.* Only *S. agona* had been demonstrated to be present in previously-collected environmental pen fecal samples from Farm B.

Mesenteric lymph nodes were collected from Farm C for 6 months. There were no samples collected in July. The overall culture prevalence for Farm C was 14%. The site-

Table 42. Summary of bacteriologic examination results of mesenteric lymph nodes collected from Farm A.

FARM	SITE	ISO NURS	DATE	+SPLS	NOSPLS	% POS	SEROTYPES
A	FINISHER	0	Aug-95	4	27	15%	*enteritidis(2), anatum(2)*
A	FINISHER	0	Sep-95	2	8	25%	*bredeney*
A	FINISHER	0	Nov-95	2	14	14%	*enteritidis*
A	FINISHER	0	Dec-95	0	40	0%	
TOTAL				8	89	9%	

ISO NURS: If there was an isolated nursery used by this farm, then the designation is 1. If there was not isolated nursery used by the farm, then the designation is 0.

+SPLS: This is the number of mesenteric lymph nodes that were positive for Salmonella by bacteriologic examination.

NOSPLS: This is the number of mesenteric lymph nodes that were collected.

Table 43. Summary of bacteriologic examination results from mesenteric lymph nodes collected at slaughter from Farm B.

FARM	SITE	ISO	DATE	+SPLS	NOSPLS	% POS	SEROTYPES
B	CONFINEMENT	1	Jul-95	0	29	0%	
B	CONFINEMENT	1	Jun-95	0	12	0%	
B	CONFINEMENT	1	Aug-95	5	28	18%	*anatum (2) typhimurium (cop) (3)*
B	CONFINEMENT	1	Sep-95	2	30	7%	*anatum, agona*
TOTAL				7	99	7%	

ISO NURS: If there was an isolated nursery used by this farm, then the designation is 1. If there was not isolated nursery used by the farm, then the designation is 0.

+SPLS: This is the number of mesenteric lymph nodes that were positive for Salmonella by bacteriologic examination.

NOSPLS: This is the number of mesenteric lymph nodes that were collected.

Table 44. Summary of the bacteriologic examination results from Farm C.

FARM	SITE	ISO NURS	DATE	NOSPLS	+SPLS	%POS	SEROTYPES
C	GREEN HILLS 11	0	Nov-95	5	55	9%	*typhimurium (2), heidelberg (2),anatum*
C	GREEN HILLS 11	0	Dec-95	15	85	18%	*anatum (2), derby (13)*
C	GREEN HILLS 9	0	Oct-95	0	2	0%	
C	I	0	Jun-95	3	30	10%	*typhimurium (3)*
C	II	0	Jun-95	1	30	3%	*heidelberg*
C	III	0	Jun-95	2	30	7%	*london, typhimurium*
C	GREEN HILLS 11	0	Jun-95	5	30	17%	*heidelberg*
C	LOCUST RIDGE 1	0	Oct-95	4	49	8%	*heidelberg (3), infantis*
C	LOCUST RIDGE 3	0	Sep-95	4	86	5%	*heidelberg (2) choleraesuis derby*
C	LOCUST RIDGE 5	0	Dec-95	3	65	5%	*choleraesuis*
C	LOCUST RIDGE 7	0	Aug-95	3	32	9%	*choleraesuis, heidelberg (2)*
C	SOMERSET F	0	Aug-95	62	90	69%	*choleraesuis (33), derby (19),heidelberg (10)*
C	SOMERSET M	0	Nov-95	0	16	0%	
C	SOMERSET P	0	Sep-95	2	29	7%	*derby choleraesuis*

Table 44. (continued)

FARM	SITE	ISO NURS	DATE	NOSPLS	+SPLS	%POS	SEROTYPES
C	SOMERSET U	0	Oct-95	6	60	10%	*typhimurium Cop, heidelberg, choleraesuis (4)*
C	SOMERSET W.5	0	Jul-95	4	112	4%	*heidelberg, typhimurium (3)*
C	SOUTH MEADOW 4	0	Aug-95	4	29	14%	*typhimurium Cop, derby(3)*
C	SOUTH MEADOW 5	0	Sep-95	0	34	0%	
C	V	0	Jun-95	9	30	30%	*choleraesuis*
C	VALLEY VIEW 2	0	Oct-95	1	59	2%	*infantis*
C	VALLEY VIEW 5.5	0	Jul-95	2	28	7%	*typhimurium*
C	VALLEY VIEW 6	0	Nov-95	8	22	36%	*typhimurium (Copenhagen), derby(7)*
C	WHITE TAIL 1	0	Nov-95	1	2	50%	*typhimurium*
C	WHITE TAIL 4	0	Aug-95	4	30	13%	*typhimurium (cop), derby(3)*
Total				148	1,035	14%	

ISO NURS: If there was an isolated nursery used by this farm, then the designation is 1. If there was not isolated nursery used by the farm, then the designation is 0.

+SPLS: This is the number of mesenteric lymph nodes that were positive for Salmonella by bacteriologic examination.

NOSPLS: This is the number of mesenteric lymph nodes that were collected.

Table 45. Summary of the *Salmonella* serotypes isolated from mesenteric lymph nodes collected from pigs at slaughter from Farm C.

Serotype	O Antigens	No. Isolates	% Isolates
S. choleraesuis	6,7	52	35%
S. derby	1, 4, 5, 12	47	32%
S. heidelberg	4, 12	27	18%
S. typhimurium (including *Copenhagen*)	1, 4, 5, 12 (1, 4, 12)	16	11%
S. anatum	3, 10	3	2%
S. infantis	6, 7	2	1%
S. london	3, 10	1	1%
Total		148	100%

specific culture prevalence ranged from 0% to 69%. The serotypes isolated and their frequency of isolation from Farm C mesenteric lymph nodes are summarized in Table 45.

Serologic examination of meat juice

Tables 46-48 contain a summary of the mix-ELISA results from the meat juice collected from muscle samples that were obtained at slaughter. Mix-ELISA results of meat juice collected from pigs from Farm A are summarized in Table 46. No samples were collected in September or October because of slaughter plant shutdowns on the days of collection. The September shutdown occurred after lymph nodes were collected but before carcasses were moved to chilling when Federal inspectors forbade the handling of the carcasses. The overall seroprevalence for Farm A was 8%. There was an increase in seroprevalence during the months of November and December.

Mix-ELISA results of meat juice collected from pigs from Farm B are summarized in Table 47. The overall seroprevalence for *Salmonella* antibodies was 15%. There was an increase in seroprevalence during the month of August (39%).

Table 46. Summary of mix-ELISA results for meat juice collected from pigs from Farm A.

FARM	SITE	ISO NURS	DATE	NO. SPLES	AVG OD%	NO. OD% >10	NO. OD% 40	%> OD%10	%> OD%40	SALEVEL
A	FINISHER	0	Aug-95	30	10.77	11	0	37%	0%	1
A	FINISHER	0	Sep-95	NONE	NONE	NONE	NONE	NONE	NONE	NONE
A	FINISHER	0	Nov-95	14	10.57	4	1	29%	7%	1
A	FINISHER	0	Dec-95	34	21.94	22	5	65%	15%	2
Total				78	15.6	37	6	47%	8%	

ISO NURS: This value is whether or not an isolated nursery was used. If an isolated nursery was used, then the designation was 1; if no isolated nursery was used, then the designation was 0.

SALEVEL: This value was determined by the seroprevalence of each group that was tested. If seroprevalence for *Salmonella* antibody was less than or equal to 10%, then SALEVEL=1. If seroprevalence was greater than 10 and less than or equal to 15%, then SALEVEL=2. If seroprevalence was greater than 15% then SALEVEL=3.

NO SPLES: This is the number of meat samples that were collected.

Table 47. Summary of mix-ELISA results from meat juice for Farm B.

FARM	SITE	ISO NURS	DATE	NO SPLES	AVG OD%	NO. OD%>10	NO. OD% >40	%> OD%10	%> OD%40	SALEVEL
B	CONFINEMENT	1	Jul-95	27	8.96	3	2	11%	7%	1
B	CONFINEMENT	1	Jun-95	27	8.96	3	2	11%	7%	1
B	CONFINEMENT	1	Aug-95	28	36.46	25	11	89%	39%	3
B	CONFINEMENT	1	Sep-95	36	15.97	18	3	50%	8%	1
TOTAL				118	17.6	49	18	42%	15%	

ISO NURS: This value is whether or not an isolated nursery was used. If an isolated nursery was used, then the designation was 1; if no isolated nursery was used, then the designation was 0.

SALEVEL: This value was determined by the seroprevalence of each group that was tested. If seroprevalence for *Salmonella* antibody was less than or equal to 10%, then SALEVEL=1. If seroprevalence was greater than 10 and less than or equal to 15%, then SALEVEL=2. If seroprevalence was greater than 15% then SALEVEL=3.

NO SPLES: This is the number of meat samples that were collected.

Mix-ELISA results of meat juice collected from pigs from Farm C are summarized in Table 48. There were no samples collected during the month of July. Sites denoted by Roman numerals were not identified to a specific site but were noted to be the same tattoo, indicating that they had come from the same site. Using an OD% cutoff of 40 for positive, there was an overall seroprevalence of *Salmonella* antibodies of 23%. There were variations in seroprevalence that are associated with season of collection.

Analysis of bacteriologic and serologic data from samples collected at slaughter

Table 49 contains a summary of all of the samples collected from the slaughter plants. Each farm was categorized based on its *Salmonella* level and it culture status.

The *Salmonella* level was determined by the seroprevalence of *Salmonella*-positive samples in each sample: *Salmonella* level 1 (less than 10%), *Salmonella* level 2 (10-15%), *Salmonella* level 3 (over 15%). The culture status was based on whether or not any samples were positive per group of mesenteric lymph nodes. There were 15 groups of pigs that were *Salmonella* level 1, 2 groups of pigs that were *Salmonella* level 2, and 13 groups of pigs that were *Salmonella* level 3. Three of the 15 *Salmonella* level 1 groups of pigs were culture-negative, both of the *Salmonella* level 2 groups of pigs were culture-negative, and there was one of the 13 *Salmonella* level 3 groups of pigs that was culture-negative.

Mesenteric lymph node culture and mix-ELISA results from pigs believed to have a low culture prevalence of *Salmonella*.

Table 50 contains a summary of the culture results from bacteriologic examination of mesenteric lymph nodes compared to the *Salmonella* level of each group of pigs from

Table 48. Summary of mix-ELISA results from meat juice for Farm C.

FARM	SITE	ISO NURS	DATE	NO. SPLES	AVG	NO. OD%>10	NO. OD% >40	%> OD%10	%> OD%40	SALEVEL
C	GREEN HILLS 11	0	Nov-95	58	4.67	7	0	12%	0%	2
C	GREEN HILLS 11	0	Dec-95	85	25.45	52	17	61%	20%	3
C	GREEN HILLS 9	0	Oct-95	2	37.00	1	1	50%	50%	3
C	I	0	Jun-95	28	41.79	23	10	82%	36%	3
C	II	0	Jun-95	28	29.54	23	8	82%	29%	3
C	III	0	Jun-95	30	45.23	27	16	90%	53%	3
C	GREEN HILLS 11	0	Jun-95	30	12.10	10	1	33%	3%	1
C	LOCUST RIDGE 1	0	Oct-95	37	7.86	8	1	22%	3%	1
C	LOCUST RIDGE 3	0	Sep-95	82	6.78	17	0	21%	0%	1
C	LOCUST RIDGE 5	0	Dec-95	65	28.66	37	16	57%	25%	3
C	LOCUST RIDGE 7	0	Aug-95	48	10.81	16	1	33%	2%	1
C	SOMERSET F	0	Aug-95	69	44.16	53	29	77%	42%	3
C	SOMERSET M	0	Nov-95	34	15.21	14	4	41%	12%	1
C	SOMERSET P	0	Sep-95	28	33.61	22	9	79%	32%	3

Table 48. (Continued)

FARM	SITE	ISO NURS	DATE	NO. SPLES	AVG	NO. OD%>10	NO. OD% >40	%> OD%10	%> OD%40	SALEVEL
C	SOMERSET U	0	Oct-95	45	34.87	31	13	69%	29%	3
C	SOMERSET W	0	Jul-95	102	61.72	99	74	97%	73%	3
C	SOUTH MEADOW 4	0	Aug-95	18	13.44	6	2	33%	11%	2
C	SOUTH MEADOW 5	0	Sep-95	31	9.06	6	2	19%	6%	1
C	V	0	Jun-95	29	49.21	26	14	90%	48%	3
C	VALLEY VIEW 2	0	Oct-95	46	5.26	5	2	11%	4%	1
C	VALLEY VIEW 5.5	0	Jul-95	47	11.55	19	3	40%	6%	1
C	VALLEY VIEW 6	0	Nov-95	4	10.25	1	0	25%	0%	1
C	WHITE TAIL 1	0	Nov-95	44	17.00	16	7	36%	16%	3
C	WHITE TAIL 4	0	Aug-95	29	5.24	5	0	17%	0%	1
TOTAL				1016	25	524	230	52%	23%	

ISO NURS: This value is whether or not an isolated nursery was used. If an isolated nursery was used, then the designation was 1; if no isolated nursery was used, then the designation was 0.

SALEVEL: This value was determined by the seroprevalence of each group that was tested. If seroprevalence for *Salmonella* antibody was less than or equal to 10%, then SALEVEL=1. If seroprevalence was greater than 10 and less than or equal to 15%, then SALEVEL=2. If seroprevalence was greater than 15% then SALEVEL=3.

NO SPLES: This is the number of meat samples that were collected.

Table 49. A summary of the results from Farms A, B, and C, culture results from mesenteric lymph nodes and *Salmonella* category. *Salmonella* level was determined by the seroprevalence of *Salmonella* antibodies for each group of pigs. *Salmonella* level 1 pigs have less than or equal to 10% seroprevalence, *Salmonella* level 2 pigs have between 10 and 15% seroprevalence, and *Salmonella* level 3, over 15%.

	Salmonella Level		
Culture Status	1	2	3
Negative	3	2	1
Positive	12	0	12
Total	15	2	13

Farm D. The *Salmonella* level for each group of pigs was determined by the seroprevalence of *Salmonella* antibodies in each group of pigs as described previously. *Salmonella* level 1 pigs differed from *Salmonella* level 3 pigs in the association between culture-positive and culture-negative mesenteric lymph nodes. Five of the 6 (83%) *Salmonella* level 3 groups of pigs were culture-positive. Two of 8 (13%) of the *Salmonella* level 1 groups of pigs were culture-positive.

Table 50. Summary of culture results from mesenteric lymph nodes and mix-ELISA results from meat juice collected from groups of pigs from Farm D. *Salmonella* level was determined by the seroprevalence of *Salmonella* antibodies for each group of pigs. *Salmonella* level 1 pigs have less than or equal to 10% seroprevalence, *Salmonella* level 2, between 10 and 15% seroprevalence, and *Salmonella* level 3, over 15%.

	Salmonella Level			
Culture Status	**1**	**2**	**3**	**Total**
Negative	6	1	1	8
Positive	2	0	5	7
Total	8	1	6	15

Mesenteric lymph node culture and mix-ELISA results from pigs from Farm C

Table 51 contains a summary of the mesenteric lymph node culture status compared to the *Salmonella* mix-ELISA status from Farm C pigs that were part of vaccine study number 4. This farm had a history of clinical salmonellosis as well as increased levels of *Salmonella* seroprevalence, based on studies presented herein. There were no seronegative groups of pigs that were also culture-negative in this sampling. Forty-four groups of pigs were classified as *Salmonella* level 3. Thirty-seven of the 44 *Salmonella* level 3 farms were

able 51. Summary of mesenteric lymph node and mix-ELISA results from samples collected at slaughter from Farm C.

	Salmonella Level			
Culture Status	**1**	**2**	**3**	**Total**
Negative	0	2	7	9
Positive	7	3	37	47
Total	7	5	44	56

culture positive. However, these results may not be valid because of unusually high OD% that was attributed to cloudy meat juice samples with particles in suspension.

Epidemiologic study number 3. Serologic survey and management survey of commercial swine farms

***Salmonella* seroprevalence studies**

A total of 9,145 samples of serum or meat juice from 267 groups of pigs from Farms A - J were tested for *Salmonella* antibody using the mix-ELISA. All samples and groups of pigs were categorized based on the season in which the samples were collected and whether or not an isolated nursery was used in the production of that group of pigs. An OD% greater than 10 was used as a cut-off for positive mix-ELISA results for individual samples. A summary of the serologic results of these samples is contained in Table 52.

There were 2,515 positive samples from the 9,145 for sample prevalence of 27.5%. The total number of groups of pigs that were positive (over 10% seroprevalence per group) for antibodies to *Salmonella* were 173. Therefore, the group seroprevalence of *Salmonella* was 65% (173/267).

Table 52. Summary of the serum samples collected from 267 farms. The column headed by the term "FARM" is the letter designation of the farms used in this study. The term "MON" is the number of the month in which samples were collected. The term "QTR" is the quarter of the year in which samples were collected. The term "NUR" is used to designate whether or not an isolated nursery was used; "0" indicated no isolated nursery used, "1" indicated that an isolated nursery was used. The term "NO." designates the number of samples collected. The columns headed by the terms "10" indicated if more than 10% (1) or if 10% or fewer (0) of the individual samples in each of the groups of pigs was positive for *Salmonella* antibody. The column headed by "40" indicates the number of samples that had an OD% greater than 40. The column headed by "%POS" is the percent of samples that were positive using an OD% cut-off of 40. The column designated by the term "LEVEL" indicates the *Salmonella* level using an OD% cut-off of 40: "1" is less than or equal to 10% positive, "2" is greater than 10 % positive but less than 15%, "3" is greater than 15% seropositive.

FARM	SORM	DATE	MON.	QTR	NUR	NO.	10	40	%POS	LEVEL
A	S	Dec-94	12	4	0	36	1	3	8%	1
A	S	Jan-95	1	1	0	30	0	1	3%	1
A	S	Feb-95	2	1	0	30	1	0	0%	1
A	S	Mar-95	3	1	0	30	1	2	7%	1
A	S	May-95	5	2	0	40	0	0	0%	1
A	S	May-95	5	2	0	49	0	0	0%	1
A	S	Jun-95	6	2	0	30	0	0	0%	1
A	S	Jul-95	7	3	0	34	0	0	0%	1
A	M	Aug-95	8	3	0	31	1	0	0%	1
A	S	Sep-95	9	3	0	57	1	4	7%	1
A	S	Oct-95	10	4	0	30	1	0	0%	1
A	M	Nov-95	11	4	0	14	1	1	7%	1
A	M	Dec-95	12	4	0	34	1	5	15%	2
B	S	Jan-95	1	1	0	30	0	0	0%	1
B	S	Feb-95	2	1	0	30	0	0	0%	1
B	S	Mar-95	3	1	1	30	1	1	3%	1
B	S	Apr-95	4	2	1	30	1	0	0%	1
B	S	May-95	5	2	1	30	0	0	0%	1
B	S	Jun-95	6	2	1	30	1	0	0%	1
B	M	Jun-95	6	2	1	26	1	2	8%	1
B	S	Jul-95	7	3	1	30	1	0	0%	1
B	M	Jul-95	7	3	1	27	1	2	7%	1
B	S	Aug-95	8	3	1	30	1	0	0%	1
B	M	Aug-95	8	3	1	28	1	11	39%	3
A	S	Aug-95	8	3	0	29	1	0	0%	1

Table 52. (continued)

FARM	SORM	DATE	MON.	QTR	NUR	NO.	10	40	%POS	LEVEL
B	S	Sep-95	9	3	1	30	0	0	0%	1
B	M	Sep-95	9	3	1	36	1	3	8%	1
B	S	Oct-95	10	4	1	30	1	0	0%	1
B	S	Nov-95	11	4	1	30	0	0	0%	1
B	S	Dec-95	12	4	1	30	0	0	0%	1
C	S	Dec-94	12	4	0	58	1	3	5%	1
C	S	Jan-95	1	1	0	30	0	0	0%	1
C	S	Jan-95	1	1	0	30	1	5	17%	3
C	S	Jan-95	1	1	0	30	0	0	0%	1
C	S	Jan-95	1	1	0	30	1	1	3%	1
C	S	Jan-95	1	1	0	30	0	0	0%	1
C	S	Feb-95	2	1	0	30	0	0	0%	1
C	S	Feb-95	2	1	0	30	1	2	7%	1
C	S	Feb-95	2	1	0	30	0	0	0%	1
C	S	Feb-95	2	1	0	30	0	0	0%	1
C	S	Feb-95	2	1	0	30	1	5	17%	3
C	S	Mar-95	3	1	0	28	1	3	11%	2
C	S	Mar-95	3	1	0	29	0	0	0%	1
C	S	Mar-95	3	1	0	29	1	3	10%	2
C	S	Mar-95	3	1	0	30	1	0	0%	1
C	S	Apr-95	4	2	0	29	0	0	0%	1
C	S	Apr-95	4	2	0	30	1	0	0%	1
C	S	Apr-95	4	2	0	29	0	0	0%	1
C	S	May-95	5	2	0	30	1	12	40%	3
C	S	May-95	5	2	0	30	1	15	50%	3
C	S	May-95	5	2	0	29	0	0	0%	1
C	S	May-95	5	2	0	32	0	0	0%	1
C	S	Jun-95	6	2	0	30	1	2	7%	1
C	S	Jun-95	6	2	0	30	0	0	0%	1
C	S	Jun-95	6	2	0	31	1	2	6%	1
C	S	Jun-95	6	2	0	30	0	1	3%	1
C	S	Jun-95	6	2	0	30	1	10	33%	3
C	M	Jun-95	6	2	0	28	1	10	36%	3
C	M	Jun-95	6	2	0	28	1	8	29%	3
C	M	Jun-95	6	2	0	30	1	16	53%	3
C	M	Jun-95	6	2	0	30	1	1	3%	1
C	M	Jun-95	6	2	0	29	1	14	48%	3
C	S	Apr-95	4	2	0	30	0	0	0%	1

Table 52. (continued)

FARM	SORM	DATE	MON.	QTR	NUR	NO.	10	40	%POS	LEVEL
C	M	Jul-95	7	3	0	102	1	74	73%	3
C	M	Jul-95	7	3	0	47	1	3	6%	1
C	S	Aug-95	8	3	0	30	1	19	63%	3
C	S	Aug-95	8	3	0	30	1	9	30%	3
C	M	Aug-95	8	3	0	48	1	1	2%	1
C	M	Aug-95	8	3	0	69	1	29	42%	3
C	M	Aug-95	8	3	0	18	1	2	11%	2
C	S	Sep-95	9	3	0	30	1	16	53%	3
C	S	Sep-95	9	3	0	30	1	14	47%	3
C	S	Sep-95	9	3	0	30	1	17	57%	3
C	S	Sep-95	9	3	0	28	1	12	43%	3
C	M	Sep-95	9	3	0	82	1	0	0%	1
C	M	Sep-95	9	3	0	28	1	9	32%	3
C	M	Sep-95	9	3	0	31	1	2	6%	1
C	M	Sep-95	9	3	0	1	1	0	0%	1
C	S	Oct-95	10	4	0	30	1	4	13%	2
C	S	Oct-95	10	4	0	30	1	10	33%	3
C	S	Oct-95	10	4	0	28	1	14	50%	3
C	M	Oct-95	10	4	0	45	1	13	29%	3
C	M	Oct-95	10	4	0	2	1	1	50%	3
C	M	Oct-95	10	4	0	37	1	1	3%	1
C	M	Oct-95	10	4	0	45	1	13	29%	3
C	M	Oct-95	10	4	0	46	1	2	4%	1
D	S	Aug-95	8	3	1	30	1	0	0%	1
D	S	Sep-95	9	3	1	30	1	0	0%	1
D	S	Sep-95	9	3	1	30	0	0	0%	1
D	S	Sep-95	9	3	1	30	1	5	17%	3
D	S	Sep-95	9	3	1	30	0	0	0%	1
D	S	Sep-95	9	3	1	30	0	0	0%	1
D	S	Sep-95	9	3	1	30	0	0	0%	1
D	S	Sep-95	9	3	1	30	1	8	27%	3
D	S	Sep-95	9	3	1	30	0	0	0%	1
D	S	Sep-95	9	3	1	30	0	1	3%	1
D	S	Sep-95	9	3	1	30	1	1	3%	1
D	S	Oct-95	10	4	1	30	0	0	0%	1
D	S	Oct-95	10	4	1	30	0	0	0%	1
D	S	Oct-95	10	4	1	30	1	6	20%	3
C	S	Nov-95	11	4	0	31	1	6	19%	3

Table 52. (continued)

FARM	SORM	DATE	MON.	QTR	NUR	NO.	10	40	%POS	LEVEL
D	S	Oct-95	10	4	1	30	1	4	13%	2
D	S	Oct-95	10	4	1	30	1	1	3%	1
D	S	Oct-95	10	4	1	30	0	4	13%	2
D	S	Oct-95	10	4	1	30	1	0	0%	1
D	S	Oct-95	10	4	1	30	0	2	7%	1
D	S	Nov-95	11	4	1	30	0	9	30%	3
D	S	Nov-95	11	4	1	30	0	0	0%	1
D	S	Nov-95	11	4	1	30	0	0	0%	1
C	S	Nov-95	11	4	0	30	1	1	3%	1
C	S	Nov-95	11	4	0	30	1	8	27%	3
C	S	Nov-95	11	4	0	26	1	10	38%	3
C	M	Nov-95	11	4	0	58	1	0	0%	1
C	M	Nov-95	11	4	0	34	1	4	12%	2
C	M	Nov-95	11	4	0	4	1	0	0%	1
C	M	Nov-95	11	4	0	44	1	7	16%	3
C	S	Dec-95	12	4	0	30	1	16	53%	3
C	S	Dec-95	12	4	0	29	0	0	0%	1
C	S	Dec-95	12	4	0	30	1	11	37%	3
C	S	Dec-95	12	4	0	28	1	17	61%	3
C	M	Dec-95	12	4	0	85	1	17	20%	3
C	M	Dec-95	12	4	0	65	1	16	25%	3
C	M	Aug-96	8	3	0	29	1	0	0%	1
D	S	Aug-95	8	3	1	30	0	0	0%	1
D	S	Aug-95	8	3	1	30	0	0	0%	1
D	S	Nov-95	11	4	1	30	1	0	0%	1
D	S	Nov-95	11	4	1	30	0	2	7%	1
D	S	Dec-95	12	4	1	29	0	0	0%	1
D	S	Dec-95	12	4	1	30	1	0	0%	1
D	S	Dec-95	12	4	1	30	0	2	7%	1
D	S	Dec-95	12	4	1	30	1	0	0%	1
D	S	Dec-95	12	4	1	30	1	0	0%	1
D	S	Dec-95	12	4	1	30	1	13	43%	3
D	S	Jan-96	1	1	1	30	0	0	0%	1
D	S	Jan-96	1	1	1	30	1	2	7%	1
D	S	Jan-96	1	1	1	30	0	0	0%	1
D	S	Nov-95	11	4	1	30	0	0	0%	1

Table 52. (continued)

FARM	SORM	DATE	MON.	QTR	NUR	NO.	10	40	%POS	LEVEL
D	S	Jan-96	1	1	1	30	0	0	0%	1
D	S	Feb-96	2	1	1	30	1	1	3%	1
D	S	Feb-96	2	1	1	30	0	0	0%	1
D	S	Feb-96	2	1	1	30	0	0	0%	1
D	S	Feb-96	2	1	1	30	1	16	53%	3
D	S	Feb-96	2	1	1	30	1	0	0%	1
D	S	Feb-96	2	1	1	30	0	0	0%	1
D	S	Feb-96	2	1	1	30	0	0	0%	1
D	S	Feb-96	2	1	1	30	0	0	0%	1
D	S	Feb-96	2	1	1	30	0	0	0%	1
D	S	Mar-96	3	1	1	30	0	0	0%	1
D	S	Mar-96	3	1	1	30	1	9	30%	3
D	S	Mar-96	3	1	1	30	0	0	0%	1
D	S	Mar-96	3	1	1	30	1	0	0%	1
D	S	Apr-96	4	2	1	30	0	1	3%	1
D	S	Apr-96	4	2	1	30	0	0	0%	1
D	S	Apr-96	4	2	1	30	0	0	0%	1
D	S	Apr-96	4	2	1	30	0	0	0%	1
D	S	May-96	5	2	1	30	0	1	3%	1
E	S	Jan-95	1	1	0	26	1	0	0%	1
E	S	Feb-95	2	1	0	53	1	2	4%	1
E	S	Aug-95	8	3	0	57	1	3	5%	1
E	S	Aug-95	8	3	0	29	0	0	0%	1
E	S	Aug-95	8	3	0	30	1	7	23%	3
E	S	Aug-95	8	3	0	30	0	0	0%	1
E	S	Aug-95	8	3	0	29	1	0	0%	1
E	S	Sep-95	9	3	0	30	1	6	20%	3
E	S	Sep-95	9	3	0	30	0	0	0%	1
E	S	Sep-95	9	3	0	30	1	0	0%	1
E	S	Sep-95	9	3	0	30	0	0	0%	1
E	S	Sep-95	9	3	0	30	1	2	7%	1
E	S	Sep-95	9	3	0	29	1	14	48%	3
E	S	Sep-95	9	3	0	30	1	1	3%	1
E	S	Oct-95	10	4	0	40	1	5	13%	2
E	S	Oct-95	10	4	0	30	1	1	3%	1
E	S	Oct-95	10	4	0	30	1	1	3%	1
E	S	Oct-95	10	4	0	30	1	5	17%	3
E	S	Oct-95	10	4	0	29	1	3	10%	2
E	S	Sep-95	9	3	0	50	0	0	0%	1

Table 52. (continued)

FARM	SORM	DATE	MON.	QTR	NUR	NO.	10	40	%POS	LEVEL
E	S	Oct-95	10	4	0	59	1	2	3%	1
E	S	Oct-95	10	4	0	30	1	1	3%	1
E	S	Nov-95	11	4	0	26	1	2	8%	1
E	S	Nov-95	11	4	0	30	1	0	0%	1
E	S	Nov-95	11	4	0	15	1	3	20%	3
E	S	Nov-95	11	4	0	14	1	0	0%	1
E	S	Nov-95	11	4	0	30	1	1	3%	1
E	S	Nov-95	11	4	0	30	1	5	17%	3
E	S	Nov-95	11	4	0	30	1	4	13%	2
E	S	Dec-95	12	4	0	30	1	0	0%	1
E	S	Dec-95	12	4	0	30	1	0	0%	1
E	S	Dec-95	12	4	0	30	1	4	13%	2
E	S	Dec-95	12	4	0	30	1	1	3%	1
E	S	Dec-95	12	4	0	30	1	0	0%	1
E	S	Dec-95	12	4	0	29	1	2	7%	1
E	S	Dec-95	12	4	0	30	1	7	23%	3
E	S	Jan-96	1	1	0	31	1	0	0%	1
E	S	Jan-96	1	1	0	30	1	1	3%	1
E	S	Jan-96	1	1	0	30	1	2	7%	1
E	S	Jan-96	1	1	0	29	1	0	0%	1
E	S	Jan-96	1	1	0	30	1	1	3%	1
E	S	Jan-96	1	1	0	30	1	0	0%	1
E	S	Jan-96	1	1	0	30	1	4	13%	2
E	S	Feb-96	2	1	0	30	1	0	0%	1
E	S	Feb-96	2	1	0	30	0	0	0%	1
E	S	Feb-96	2	1	0	30	1	0	0%	1
E	S	Feb-96	2	1	0	30	1	6	20%	3
E	S	Feb-96	2	1	0	30	1	2	7%	1
E	S	Feb-96	2	1	0	30	1	3	10%	1
E	S	Feb-96	2	1	0	30	1	6	20%	3
E	S	Mar-96	3	1	0	30	0	1	3%	1
E	S	Mar-96	3	1	0	30	0	0	0%	1
E	S	Mar-96	3	1	0	30	1	4	13%	2
E	S	Mar-96	3	1	0	30	1	2	7%	1
E	S	Mar-96	3	1	0	30	1	0	0%	1
E	S	Mar-96	3	1	0	30	1	0	0%	1
E	S	Mar-96	3	1	0	30	1	0	0%	1
E	S	Mar-96	3	1	0	30	0	0	0%	1
E	S	Feb-96	2	1	0	30	0	0	0%	1

Table 52. (continued)

FARM	SORM	DATE	MON.	QTR	NUR	NO.	10	40	%POS	LEVEL
E	S	Apr-96	4	2	0	30	1	0	0%	1
E	S	Apr-96	4	2	0	30	0	0	0%	1
E	S	Apr-96	4	2	0	22	1	0	0%	1
E	S	Apr-96	4	2	0	30	1	6	20%	3
E	S	Apr-96	4	2	0	30	0	0	0%	1
E	S	Apr-96	4	2	0	30	1	0	0%	1
E	S	Apr-96	4	2	0	30	0	0	0%	1
E	S	May-96	5	2	0	30	1	0	0%	1
E	S	May-96	5	2	0	30	1	0	0%	1
E	S	May-96	5	2	0	30	0	0	0%	1
E	S	May-96	5	2	0	30	1	1	3%	1
E	S	May-96	5	2	0	30	1	2	7%	1
E	S	May-96	5	2	0	19	1	1	5%	1
F	S	Jan-95	1	1	1	30	1	5	17%	3
F	S	Jan-95	1	1	1	30	0	0	0%	1
F	S	Feb-95	2	1	1	30	0	0	0%	1
F	S	Feb-95	2	1	1	29	0	0	0%	1
F	S	Mar-95	3	1	1	30	1	4	13%	2
F	S	Mar-95	3	1	1	30	1	7	23%	3
F	S	Apr-95	4	2	1	29	0	0	0%	1
F	S	Apr-95	4	2	1	30	0	0	0%	1
F	S	Jun-95	6	2	1	30	0	0	0%	1
F	S	Jun-95	6	2	1	30	0	0	0%	1
F	M	Aug-95	8	3	1	44	1	0	0%	1
F	M	Sep-95	9	3	1	28	1	0	0%	1
F	M	Sep-95	9	3	1	29	1	1	3%	1
F	M	Oct-95	10	4	1	32	1	1	3%	1
F	M	Oct-95	10	4	1	31	1	2	6%	1
F	M	Nov-95	11	4	1	30	0	1	3%	1
F	M	Nov-95	11	4	1	30	0	0	0%	1
G	S	Jan-95	1	1	1	43	0	0	0%	1
G	S	Feb-95	2	1	1	38	0	1	3%	1
G	S	Apr-95	4	2	1	28	0	0	0%	1
G	S	May-95	5	2	1	38	0	0	0%	1
G	S	Sep-95	9	3	1	38	0	0	0%	1
G	S	Oct-95	10	4	1	41	0	0	0%	1
G	S	Nov-95	11	4	1	40	0	0	0%	1
G	S	Nov-95	11	4	1	30	0	0	0%	1
H	S	Jan-95	1	1	1	60	1	5	8%	1

Table 52. (continued)

FARM	SORM	DATE	MON.	QTR	NUR	NO.	10	40	%POS	LEVEL
H	S	Feb-95	2	1	1	57	1	4	7%	1
H	S	Mar-95	3	1	1	57	1	2	4%	1
H	S	Apr-95	4	2	1	59	1	1	2%	1
H	S	May-95	5	2	1	59	1	0	0%	1
H	S	Jun-95	6	2	1	48	1	1	2%	1
H	S	Jul-95	7	3	1	60	1	0	0%	1
H	S	Aug-95	8	3	1	59	1	0	0%	1
H	S	Sep-95	9	3	1	60	1	0	0%	1
H	S	Oct-95	10	4	1	60	1	0	0%	1
H	S	Nov-95	11	4	1	59	1	0	0%	1
H	S	Dec-95	12	4	1	60	1	0	0%	1
I	S	Nov-94	11	4	1	60	0	2	3%	1
J	S	Mar-95	3	1	0	59	0	0	0%	1

Table 53 contains a comparison of the mean OD% of pigs that were reared in an isolated nursery with the mean OD% of pigs that were not reared in an isolated nursery. The mean OD% of pigs that were reared in isolated nurseries was significantly ($p < .0001$) less than the mean OD% of pigs that were not reared in isolated nurseries.

Table 53. A comparison of the mean OD% of serum or meat juice samples collected from pigs that were reared in an isolated nursery with the mean OD% of serum or meat juice samples collected from pigs that were not reared in an isolated nursery.

Isolated Nursery	n	Mean OD%	s.d.	s.e.	95% C.I.
No	5191	15.01	24.1	.33	14.4 - 15.7
Yes	3953	7.7*	16.7	.27	7.2 - 8.2

***Mean difference is significant, p < .0001**

Table 54 contains a comparison of the *Salmonella* seroprevalence in pigs that were reared in isolated nurseries and those pigs that were not reared in isolated nurseries. The pigs that were reared in isolated nurseries had a significantly lower seroprevalence of *Salmonella* antibody.

Table 54. A comparison of the *Salmonella* seroprevalence in pigs that were reared in an isolated nursery with *Salmonella* seroprevalence in pigs that were not reared in an isolated nursery.

Isolated Nursery	n	Sero-prevalence	s.d.	s.e.	95% C.I.
No	5191	0.34	.47	.01	.32 - .35
Yes	3953	0.19*	.39	.01	.18 - .20

***Mean difference is significant, p <.0001.**

Table 55 contains a summary of the differences in mean OD% observed for each quarter of the year in which the samples were collected. The mean OD% for the samples that were collected in the quarter of July through September were significantly higher than the samples collected in any of the other three quarters of the year. In addition, mean OD% of the samples collected during the quarter of October through December were higher than the mean OD% of the samples collected in the quarters of January through March and April through June.

Table 55. A comparison of the mean OD% of serum or meat juice samples collected from pigs during the four different quarters of the year.

Quarter	n	Mean OD%	s.d.	s.e.	95% C.I.
Jan-Mar	2429	9.31[a]	19.00	.3856	8.56 -10.06
Apr-Jun	2367	11.14[b]	20.92	.4299	10.30 - 11.99
Jul-Sep	1369	16.27[c]	25.58	.6916	14.92 - 17.63
Oct-Dec	2979	12.44[b]	21.81	.3996	11.65 - 13.22

[a,b,c] means with different superscripts are significantly different, $p < .05$.

The seroprevalence of *Salmonella* antibodies was also compared for each of the quarters in which the samples were collected. The results of this comparison are contained in Table 56. The quarter of July through September had the highest seroprevalence of *Salmonella* antibodies and was significantly different than any of the other three quarters. The seroprevalence of *Salmonella* antibodies for the quarter of October through December was higher than the quarters of January through march and April through June.

Table 56. A comparison of the seroprevalence of *Salmonella* in pigs during the four different quarters of the year.

Quarter	n	Sero-prevalence	s.d.	s.e.	95% C.I.
Jan-Mar	2429	.2194[a]	.4139	.0084	.20 - .24
Apr-Jun	2367	.2581[b]	.4377	.0090	.24 - .28
Jul-Sep	1369	.3440[c]	.4752	.0128	.32 - .37
Oct-Dec	2979	.3011[d]	.4588	.0084	.28 - .32

[a,b,c,d] means with different superscripts are significantly different, $p < .05$.

Table 57 contains a summary of the mean OD% for each nursery management method (isolated nursery used versus no isolated nursery used) and for each season in which the samples were collected. Table 58 contains a summary of the seroprevalence for each quarter for the two different nursery management methods (isolated nursery used or no isolated nursery used). Table 59 contains the odds ratio for the likelihood of *Salmonella* seropositive for each season of the year in which samples were collected.

Table 57. Summary of the mean OD% for each season and nursery management type.

	Isolated Nursery	
	No	Yes
Quarter	(n)	(n)
Jan-Mar	9.76	8.63
	(1456)	(973)
Apr-Jun	15.04	4.88
	(1460)	(907)
Jul-Sep	23.73	6.94
	(761)	(608)
Oct-Dec	15.67	9.10
	(1514)	(1465)

Table 58. Summary of seroprevalence of *Salmonella* antibodies by nursery management method and by season.

	Isolated nursery used	
	No	Yes
Quarter	(n)	(n)
Jan-Mar	.23	.20
	(1456)	(973)
Apr-Jun	.33	.15
	(1460)	(907)
Jul-Sep	.49	.17
	(761)	(608)
Oct-Dec	.38	.22
	(1514)	(1465)

Table 59. Odds ratio (OR, with 95% confidence interval) calculation for each quarter in which samples were tested for antibody to *Salmonella*. The columns that are headed "A", "B", "C", and "D" represent the quadrants of a two-by-two table. The column designated "VAR" contains the estimate of the variance of the values in the two-by-two table.

QTR	A	B	C	D	VAR	OR	95% C.I.	
1	533	1896	1979	4736	0.0031	0.67	0.60	0.75
2	611	1756	1901	4876	0.0029	0.89	0.80	0.99
3	471	898	2041	5734	0.0039	1.47	1.30	1.67
4	897	2082	1615	4550	0.0024	1.21	1.10	1.34

In all of the quarters of the year, pigs that were reared in isolated nurseries had lower seroprevalence of *Salmonella* antibody and a lower OD% when tested for *Salmonella* antibody. These results indicate that pigs that are near slaughter weight in the first two quarters of the year (January through March, and April through June) tended to have a significantly lower seroprevalence of *Salmonella* antibody. This was in contrast to pigs that were near slaughter weight in the last two quarters of the year (July through September and October through December) which had a significantly higher seroprevalence in those quarters.

Table 60 contains a summary of the data from these farms when the differences in seasonal effects are examined for pigs that were not reared in an isolated nursery. These results indicate that the seasonal effects seen in Table 59 also appear when there is no isolated nursery used to raise pigs. Table 61 contains a summary of the data from these farms when the differences in seasonal effects are examined for pigs that were reared in an isolated nursery. There appears to be a tendency to have reduced seroprevalence in quarter 2 and increased seroprevalence in quarter 4 regardless of nursery management used.

Table 60. Odds ratio (OR, with 95% confidence interval) calculation for each quarter in which samples from pigs that were not reared in an isolated nursery and were tested for antibody to *Salmonella*. The columns that are headed "A", "B", "C", and "D" represent the quadrants of a two-by-two table. The column designated "VAR" contains the estimate of the variance of the values in the two-by-two table.

QTR	A	B	C	D	VAR	OR	95% C.I.
1	341	1115	1426	2309	0.0050	0.50	0.43 - 0.57
2	479	981	1288	2443	0.0043	0.93	0.81 - 1.05
3	370	391	1397	3033	0.0063	2.05	1.76 - 2.40
4	577	937	1190	2487	0.0040	1.29	1.14 - 1.46

When the odds ratio was calculated for the seroprevalence differences between pigs reared in an isolated facility and pigs not reared in an isolated nursery in the third quarter, pigs that were reared without an isolated nursery were 4.75 times (95% C.I., 3.67,6.14) more likely to be positive for *Salmonella.*

Table 61. Odds ratio (OR, with 95% confidence interval) calculation for each quarter in which samples from pigs that were reared in an isolated nursery and were tested for antibody to *Salmonella.* The columns that are headed "A", "B", "C", and "D" represent the quadrants of a two-by-two table. The column designated "VAR" contains the estimate of the variance of the values in the two-by-two table.

QTR	A	B	C	D	VAR	OR	95% C.I.	
1	192	781	553	2427	0.0087	1.08	0.90	1.30
2	132	775	613	2433	0.0109	0.68	0.55	0.83
3	101	507	644	2701	0.0138	0.84	0.66	1.05
4	320	1145	425	2063	0.0068	1.36	1.15	1.60

Table 62 contains a summary of each of the 267 groups of pigs that were tested for antibody to *Salmonella* and categorized according to the Danish method of assigning a *Salmonella* level based on seroprevalence. As seen with the distribution of seroprevalence by individual sample, there appears to be a tendency for an increase in the number of *Salmonella*-positive herds to occur in the last two quarters of the year. In the Jan-Mar quarter, the percent of positive (level 2 and 3) groups of pigs was 17.6% (13/74), 15.4% (8/52) for Apr-Jun, 27.1% (16/59) for Jul-Sep, and 37.6% (30/82) for Oct-Dec.

Table 62. Comparison of seasonal distribution of groups of pigs according to *Salmonella* seroprevalence. *Salmonella* level 1 groups have a seroprevalence of less than 10%, level 2 groups have a seroprevalence of greater than or equal to 10% and less than or equal to 15%, level 3 groups have a seroprevalence of greater than 15%.

	Salmonella Level		
Quarter	**1**	**2**	**3**
Jan-Mar	61	5	8
Apr-Jun	44	0	8
Jul-Sep	43	1	15
Oct-Dec	52	9	21

Table 63 contains a summary of the seroprevalence (*Salmonella* level) for each of the 267 groups of pigs and grouped according to whether or not there was an isolated nursery used to rear the pigs in their respective groups. There is a tendency for a higher proportion of groups of pigs that were not reared in an isolated nursery to be positive for *Salmonella* antibodies.

There was a higher proportion of groups of pigs reared without an isolated nursery that were *Salmonella*-positive. There were 12.2% (13/107) of the groups of pigs that were

Table 63. Comparison of nursery management system (isolated nursery versus no isolated nursery) groups of pigs according to *Salmonella* seroprevalence. *Salmonella* level 1 groups have a seroprevalence of less than 10%, level 2 groups have a seroprevalence of greater than or equal to 10% and less than or equal to 15%, level 3 groups have a seroprevalence of greater than 15%.

	Salmonella Level		
Isolated Nursery	**1**	**2**	**3**
No	106	12	42
Yes	94	3	10

reared in isolated nurseries that were positive for *Salmonella* (level 2 and level 3). There were 33.8% (54/160) of the groups of pigs that were not reared in isolated nurseries that were positive for *Salmonella*.

***Yersinia, Toxoplasma*, and *Trichinella* serology**

There were 4,077 samples of serum collected 124 groups of pigs that were tested for the presence of antibody to *Y. enterocolitica* O:3. The results of the *Y. enterocolitica* ELISA results are summarized in Table 64 along with the *Salmonella* mix-ELISA results from the same group of pigs that was tested. The sample prevalence of *Y. enterocolitica* O:3 was 38%. The group prevalence was 60.2%. Each group of pigs that was tested fell into one of three categories, based on seroprevalence: negative (0% of samples with OD% > 10), low (3.0-6.0% of samples with OD% >10), or high (greater than 10% of the samples with OD%>10).

When the results from all of the individual *Yersinia* results were compared with paired sample *Salmonella* serology results, there was a slight but significant correlation between pair-wise samples using Pearson (Corr. Coef. = .0899, $p < .0001$) and Spearman correlation models (Corr. Coef. = .3066, $p < .0001$). When farms were compared, assigning a positive/negative status for *Y. enterocolitica* O:3 and *Salmonella*, there tended to be an association between farms that were positive for antibody to *Salmonella* and positive for antibody for *Y. enterocolitica* O:3. The correlation coefficient for the ordinal data of farms was.4391 ($p < .001$). Figures 4 and 5 describe show graphs of the sample and herd prevalence, respectively, for *Y. enterocolitica* O:3 and *Salmonella*.

Table 64. Summary of the *Y. enterocolitica* O:3 ELISA results for the farms that were tested. A summary of the *Salmonella* mix-ELISA results is included for each group of pigs that was tested for *Y. enterocolitica* O:3. "FARM" is the same farm designation that was used throughout the epidemiologic studies. "SALSPLES" and "YERSPLE" are the number of samples tested for *Salmonella* and *Y. enterocolitica* O:3, respectively. "SALPOS" and YERSPOS" are the number of samples that were positive when tested for *Salmonella* and *Y. enterocolitica* O:3, respectively. An OD% of greater than 10 was used as a cut-off for positive values for both ELISA results. "SALPREV" and YERSPREV" are the sample seroprevalence values for *Salmonella* and *Y. enterocolitica* O:3, respectively. "SALSTAT" and YERSSTAT" are the categorical designations for *Salmonella* and *Y. enterocolitica* O:3 group status, respectively. A group of pigs was considered to be positive for either pathogen if the group seroprevalence was greater than 10%.

FARM	DATE	MONTH	SAL SPLES	SAL POS	SAL PREV	SAL STAT	YER SPLE	YER SPOS	YERS PREV	YERS STAT	SAL STAT
B	Jan-95	1	30	1	3%	0	30	0	0%	0	0
C	Jan-95	1	30	0	0%	0	30	0	0%	0	0
C	Jan-95	1	30	0	0%	0	30	11	37%	1	0
C	Jan-95	1	30	12	40%	1	30	0	0%	0	1
C	Jan-95	1	30	6	20%	1	30	16	53%	1	1
E	Jan-95	1	37	7	19%	1	37	14	38%	1	1
E	Jan-95	1	18	7	39%	1	18	2	11%	1	1
D	Jan-96	1	30	2	7%	0	30	14	47%	1	0
E	Jan-96	1	30	14	47%	1	30	29	97%	1	1
E	Jan-96	1	11	6	55%	1	11	11	100%	1	1
E	Jan-96	1	8	0	0%	0	8	4	50%	1	0
E	Jan-96	1	30	12	40%	1	30	30	100%	1	1
C	Feb-95	2	30	0	0%	0	30	1	3%	0	0
C	Feb-95	2	30	11	37%	1	30	9	30%	1	1
C	Feb-95	2	30	0	0%	0	30	1	3%	0	0
E	Feb-95	2	30	15	50%	1	30	20	67%	1	1
E	Feb-95	2	26	8	31%	1	26	5	19%	1	1
D	Feb-96	2	30	7	23%	1	30	13	43%	1	1
E	Feb-96	2	30	4	13%	1	30	26	87%	1	1
E	Feb-96	2	30	0	0%	0	27	3	11%	1	0
E	Feb-96	2	30	6	20%	1	30	27	90%	1	1
C	Jan-95	1	30	1	3%	0	30	0	0%	0	0

Table 64. (continued)

FARM	DATE	MONTH	SAL SPLES	SAL POS	SAL PREV	SAL STAT	YER SPLE	YER SPOS	YERS PREV	YERS STAT	SAL STAT
E	Feb-96	2	30	27	90%	1	30	15	50%	1	1
E	Feb-96	2	30	3	10%	0	30	16	53%	1	0
E	Feb-96	2	30	6	20%	1	30	2	7%	0	1
E	Feb-96	2	30	10	33%	1	30	26	87%	1	1
E	Feb-96	2	30	22	73%	1	30	30	100%	1	1
C	Mar-95	3	29	8	28%	1	27	17	63%	1	1
F	Mar-95	3	30	2	7%	0	30	30	100%	1	0
F	Mar-95	3	30	0	0%	0	30	0	0%	0	0
D	Mar-96	3	30	0	0%	0	30	0	0%	0	0
E	Mar-96	3	30	2	7%	0	30	17	57%	1	0
E	Mar-96	3	30	2	7%	0	30	30	100%	1	0
E	Mar-96	3	30	25	83%	1	30	14	47%	1	1
E	Mar-96	3	30	13	43%	1	30	24	80%	1	1
E	Mar-96	3	30	6	20%	1	30	30	100%	1	1
E	Mar-96	3	30	6	20%	1	30	28	93%	1	1
E	Mar-96	3	30	7	23%	1	30	28	93%	1	1
E	Mar-96	3	30	2	7%	0	23	19	83%	1	0
E	Apr-96	4	7	0	0%	0	7	6	86%	1	0
A	Aug-95	8	29	3	10%	1	19	19	100%	1	1
C	Aug-95	8	19	6	32%	1	22	1	5%	0	1
F	Aug-95	8	45	9	20%	1	48	1	2%	0	1
F	Aug-95	8	29	3	10%	1	31	5	16%	1	1
A	Sep-95	9	57	21	37%	1	57	0	0%	0	1
B	Sep-95	9	36	17	47%	1	38	22	58%	1	1
C	Sep-95	9	79	16	20%	1	83	69	83%	1	1
C	Sep-95	9	38	7	18%	1	29	1	3%	0	1
C	Sep-95	9	26	23	88%	1	26	8	31%	1	1
D	Sep-95	9	30	6	20%	1	30	0	0%	0	1
D	Sep-95	9	30	3	10%	0	30	0	0%	0	0
D	Sep-95	9	30	0	0%	0	30	0	0%	0	0
D	Sep-95	9	30	3	10%	0	30	0	0%	0	0
D	Sep-95	9	30	6	20%	1	30	0	0%	0	1
D	Sep-95	9	30	2	7%	0	30	0	0%	0	0
F	Sep-95	9	28	4	14%	1	30	16	53%	1	1
A	Aug-95	8	33	11	33%	1	31	15	48%	1	1

Table 64. (continued)

FARM	DATE	MONTH	SAL SPLES	SAL POS	SAL PREV	SAL STAT	YER SPLE	YER SPOS	YERS PREV	YERS STAT	SAL STAT
A	Oct-95	10	30	4	13%	1	30	20	67%	1	1
B	Oct-95	10	30	8	27%	1	24	0	0%	0	1
C	Oct-95	10	30	13	43%	1	30	13	43%	1	1
C	Oct-95	10	30	25	83%	1	30	14	47%	1	1
C	Oct-95	10	27	26	96%	1	27	5	19%	1	1
D	Oct-95	10	30	11	37%	1	30	0	0%	0	1
D	Oct-95	10	30	16	53%	1	30	30	100%	1	1
D	Oct-95	10	30	3	10%	0	30	2	7%	0	0
D	Oct-95	10	30	14	47%	1	30	0	0%	0	1
D	Oct-95	10	30	2	7%	0	30	0	0%	0	0
D	Oct-95	10	30	3	10%	0	30	0	0%	0	0
D	Oct-95	10	30	2	7%	0	30	1	3%	0	0
D	Oct-95	10	30	2	7%	0	30	0	0%	0	0
D	Oct-95	10	30	21	70%	1	30	30	100%	1	1
E	Oct-95	10	30	9	30%	1	30	21	70%	1	1
E	Oct-95	10	30	8	27%	1	30	14	47%	1	1
E	Oct-95	10	30	7	23%	1	30	4	13%	1	1
E	Oct-95	10	29	8	28%	1	30	28	93%	1	1
E	Oct-95	10	40	25	63%	1	40	35	88%	1	1
E	Oct-95	10	40	13	33%	1	40	27	68%	1	1
F	Oct-95	10	30	6	20%	1	30	0	0%	0	1
G	Oct-95	10	39	0	0%	0	39	0	0%	0	0
B	Nov-94	11	70	9	13%	1	146	0	0%	0	1
SC	Nov-94	11	60	0	0%	0	70	0	0%	0	0
A	Nov-95	11	14	5	36%	1	14	2	14%	1	1
C	Nov-95	11	29	2	7%	0	29	19	66%	1	0
C	Nov-95	11	31	19	61%	1	31	1	3%	0	1
C	Nov-95	11	30	9	30%	1	30	0	0%	0	1
C	Nov-95	11	122	38	31%	1	130	80	62%	1	1
D	Nov-95	11	30	11	37%	1	30	0	0%	0	1
D	Nov-95	11	30	2	7%	0	30	0	0%	0	0
D	Nov-95	11	30	0	0%	0	30	0	0%	0	0
D	Nov-95	11	30	0	0%	0	30	0	0%	0	0
D	Nov-95	11	30	0	0%	0	30	0	0%	0	0
E	Nov-95	11	30	6	20%	1	30	21	70%	1	1
E	Oct-95	10	30	13	43%	1	30	26	87%	1	1

Table 64. (continued)

FARM	DATE	MONTH	SAL SPLES	SAL POS	SAL PREV	SAL STAT	YER SPLE	YER SPOS	YERS PREV	YERS STAT	SAL STAT
E	Nov-95	11	26	15	58%	1	26	26	100%	1	1
E	Nov-95	11	30	8	27%	1	30	20	67%	1	1
E	Nov-95	11	14	3	21%	1	15	14	93%	1	1
E	Nov-95	11	30	25	83%	1	30	17	57%	1	1
E	Nov-95	11	30	114	380%	1	30	3	10%	0	1
F	Nov-95	11	30	4	13%	1	30	29	97%	1	1
F	Nov-95	11	16	0	0%	0	16	0	0%	0	0
A	Dec-94	12	62	11	18%	1	36	0	0%	0	1
C	Dec-94	12	97	11	11%	1	60	8	13%	1	1
H	Dec-94	12	36	4	11%	1	97	0	0%	0	1
SC	Dec-94	12	30	14	47%	1	62	0	0%	0	1
C	Dec-95	12	29	24	83%	1	27	3	11%	1	1
C	Dec-95	12	30	32	107%	1	30	3	10%	0	1
C	Dec-95	12	30	0	0%	0	30	1	3%	0	0
C	Dec-95	12	30	26	87%	1	30	21	70%	1	1
D	Dec-95	12	30	30	100%	1	30	29	97%	1	1
D	Dec-95	12	30	2	7%	0	30	30	100%	1	0
D	Dec-95	12	30	2	7%	0	30	0	0%	0	0
D	Dec-95	12	30	4	13%	1	30	30	100%	1	1
D	Dec-95	12	30	0	0%	0	30	0	0%	0	0
D	Dec-95	12	30	5	17%	1	30	0	0%	0	1
E	Dec-95	12	30	7	23%	1	30	2	7%	0	1
E	Dec-95	12	30	7	23%	1	30	9	30%	1	1
E	Dec-95	12	30	6	20%	1	30	30	100%	1	1
E	Dec-95	12	30	25	83%	1	30	19	63%	1	1
E	Dec-95	12	30	12	40%	1	30	30	100%	1	1
E	Dec-95	12	30	20	67%	1	30	23	77%	1	1
E	Dec-95	12	30	11	37%	1	30	25	83%	1	1
G	Dec-95	12	30	0	0%	0	30	29	97%	1	0
H	Dec-95	12	60	14	23%	1	60	49	82%	1	1

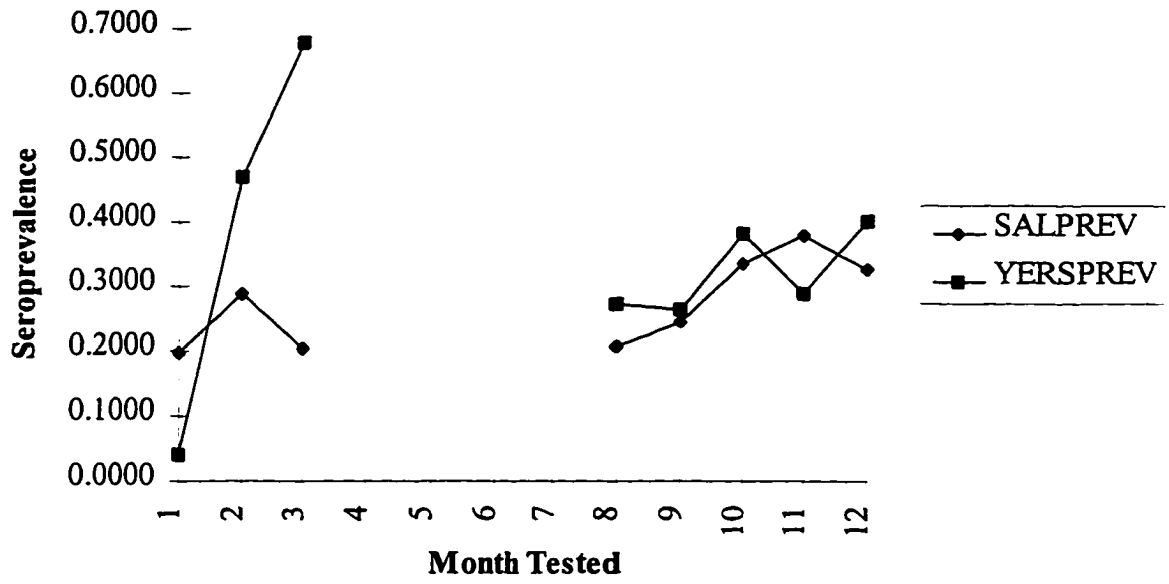

Figure 4. Comparison of mix ELISA results of sample prevalence from herds that were tested for *Salmonella* antibody and *Y. enterocolitica* O:3 antibody. Sample prevalence for both pathogens is plotted for each month from which the samples were taken. That is, Month 1 is January , Month 2, February, and so on.

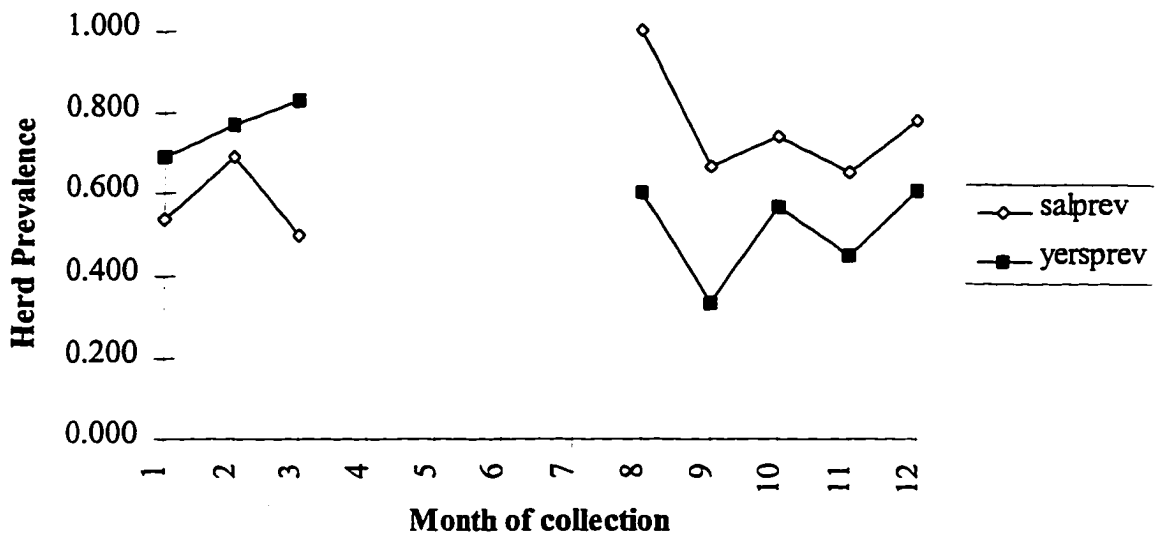

Figure 5. Comparison of mix ELISA results of herd seroprevalence of *Y. enterocolitica* O:3 and *Salmonella*. Herd prevalence is plotted against the month from which the sample was taken. That is, Month 1 is January, Month 2, February, and so on.

There were 2,140 samples of serum collected from 72 groups of that were tested for the presence of antibody to *T. spiralis*. Only 3 samples, each from a different group of pigs were positive. Sample prevalence was 0.14% while the group prevalence was 4.2%.

There were 1,812 samples of serum collected from 61 groups of pigs that were tested for the presence of antibody to *T. gondii*. Only 2 samples from 2 different groups of pigs were positive. The sample prevalence was 0.11% and the group prevalence was 3.3% .

Comparison of results from questionnaire. A questionnaire was completed and received for 89 of the 267 groups of pigs mentioned above. These questionnaires were completed for Farms A, B, C, F, G, and H. There were 48 of the questionnaires that included pigs, feed, and death loss data for analysis of biological performance. The data obtained in these 48 came from Farms C, F, and H. The data from these questionnaires is presented in Table 65.

Appendix 1 contains a summary of the factors that were part of the questionnaire, the risk factor analysis for the factor that was chosen for analysis and believed to be associated with a *Salmonella* seroprevalence greater than 10% using an OD% cut-off of 40 (which would be a level 2 or level 3 herd in the Danish method of grading herds), their respective odds ratio, and the 95% confidence interval for each factor. Those factors which appeared to be associated with high seroprevalence of *Salmonella* were: Audit score greater than 10, no isolated nursery, failure to remove deads daily, feed produced by farm personnel, using a dry feed feeding system, feeding pellets instead of meal, taking longer than one day to fill a finishing site or building, failure to adhere, to all-in-all-out pig flow,

Table 65. Risk factor analysis from information obtained from questionnaire. Columns designated "A", "B", "C", and "D" correspond to the quadrants in a two-by-two table. "OR" is the calculated odds ratio. Factors with an asterisk (*) had a cell value of 0; 1 was then added to each value in the quadrants. "VAR" is the estimated variance of the cell ("A", "B", "C", and "D") values.

FACTOR	A	B	C	D	VAR	OR	95% C.I.	
Audita >=10	17	54	6	12	0.3273	0.63	0.21	1.93
Audita >= 7	20	59	3	7	0.5431	0.79	0.19	3.36
Audts >=7	21	41	4	25	0.3620	3.20	0.98	10.42
No bait*	1	24	24	44	1.1061	0.08	0.01	0.60
No boot bath*	1	12	24	56	1.1429	0.19	0.02	1.58
Not bird proofed	1	12	24	56	1.1429	0.19	0.02	1.58
No boot change	20	29	3	27	0.4549	6.21	1.65	23.30
Not cleaned before*	1	6	24	62	1.2245	0.43	0.05	3.77
Deads not removed daily	19	18	4	48	0.3790	12.67	3.79	42.37
Not disinfected*	1	12	23	56	1.1447	0.20	0.02	1.65
Feed ground on farm	24	57	1	13	1.1361	5.47	0.68	44.28
Dry feed	19	40	4	26	0.3661	3.09	0.94	10.12
Pellets fed	19	17	4	49	0.3819	13.69	4.07	46.00
More than one fill day	20	42	3	24	0.4488	3.81	1.02	14.17
More than one week fill	18	41	5	25	0.3199	2.20	0.72	6.66
No slotted floor*	1	14	24	54	1.1316	0.16	0.02	1.29
Continuous flow	10	24	13	42	0.2424	1.35	0.51	3.54
Two-site	19	30	4	36	0.3637	5.70	1.75	18.60
Holdbacks	11	29	12	37	0.2358	1.17	0.45	3.03
Hose in finisher*	1	12	24	55	1.1432	0.19	0.02	1.55
No isolated nursery	19	30	4	36	0.3637	5.70	1.75	18.60
Recycled flush water	19	17	4	20	0.4115	5.59	1.59	19.66
More than 1 nursery source	19	18	4	48	0.3790	12.67	3.79	42.37
No nozzle on hose	19	28	4	38	0.3647	6.45	1.97	21.07
First qtr fill	3	16	20	50	0.4658	0.47	0.12	1.79
Second qtr fill	8	11	15	55	0.3008	2.67	0.91	7.82
Third qtr fill	8	17	15	49	0.2709	1.54	0.55	4.27
Fourth qtr fill	4	22	19	44	0.3708	0.42	0.13	1.39
No sep. trailer for sales*	1	24	24	44	1.1061	0.08	0.01	0.60
Employees shower*	1	24	24	44	1.1061	0.08	0.01	0.60
Visitors shower*	1	24	24	44	1.1061	0.08	0.01	0.60
Transport personnel in bldg	22	32	1	34	1.1061	23.38	2.97	183.89
Trailer cleaned after*	1	24	24	44	1.1061	0.08	0.01	0.60
Trailer cleaned before*	1	24	24	44	1.1061	0.08	0.01	0.60
Mechanical ventilation*	1	24	24	44	1.1061	0.08	0.01	0.60

use of recycled lagoon water (compared to fresh water) for manure removal, more than one nursery used to fill a building or a site, no nozzled boot hose, and placement of pigs during the quarter of Apr-Jun and the quarter of Jul-Sep.

A comparison was made of the differences in feed efficiency at the 3 different *Salmonella* levels for the 48 groups of pigs that submitted these data. Level 3 groups of pigs had the best feed conversion but the difference was not statistically significant ($p < .05$).

The pounds of live pig produced per square foot of finishing building per year was compared for the three different *Salmonella* levels. Table 66 contains a summary of that analysis. *Salmonella* level 1 groups of pigs had a mean production of 68.9 pound of live pig produced per square foot of building per year compared to *Salmonella* level 3 groups of pigs that had a mean production of 63.7 pounds of live pig produced per square foot of building per year.

Table 66. Comparison of the pounds of live pig produced per square foot of building per year among the different *Salmonella* levels. *Salmonella* level 1 groups have a seroprevalence less than or equal to 10%, level 2 greater than 10% but less than or equal to 15%; *Salmonella* level 3 groups have s seroprevalence greater than 15%

Salmonella Level	n	Mean Pounds*	s.d.	s.e.	95% C.I.
1	28	68.9[a]	4.38	.83	67.2 - 70.6
2	3	66.0	1.38	.79	62.6 - 69.5
3	17	63.7[b]	3.67	.89	61.8 - 65.9

*mean pounds of live pig per square foot of building per year
[a,b] column difference was significant, $p = .0006$.

CHAPTER FIVE. DISCUSSION

Vaccine studies

The results from vaccine study number one show that vaccination of pigs at one day of age with SC54™ will protect pigs against challenge with *S. choleraesuis*. After pigs were challenged with *S. choleraesuis*, all three treatment groups developed clinical signs on the day after challenge. However, by the second day post-challenge, clinical signs for the pigs that were vaccinated at one day of age began to return to normal. Throughout the study, the severity of the change in clinical signs observed in the pigs that were vaccinated at one day of age was less than either of the other two treatment groups. The clinical signs of pigs that were vaccinated at 21 d of age also tended to return to normal after challenge. One pig from the group of pigs vaccinated at 21 d of age died of polyserositis. Although polyserositis is not a typical lesion associated with paratyphoid, *S. choleraesuis* was isolated from this pig. The death of this one pig contributed to a major part of the increase in observed clinical signs of treatment group number 2. The increase in average clinical score for group 3 was high because of the high death loss (7 of 10 pigs died after challenge) observed in this group. These trends in clinical response are similar to those reported previously in safety and efficacy studies of this vaccine when used in 21 d old pigs (Roof and Doitchinoff 1995).

Pigs that were vaccinated at one day of age or at 21 days of age developed an increased rectal temperature after challenge. However, both of the vaccinated groups of pigs had significantly lower mean rectal temperatures than nonvaccinated pigs for the course of the study after challenge with *S. choleraesuis*. These results are also in agreement with those reported previously by Roof and Doitchinoff (1995). Pigs vaccinated at one day of age had a

lower mean rectal temperature following challenge when compared to either of the other two treatment groups. When daily mean rectal temperatures are compared, all three treatment groups developed elevated mean rectal temperatures on the day after challenge (day 1). While pigs in treatment groups 2 and 3 tended to have the similar apparent rates of increase following challenge, mean rectal temperature of pigs in treatment group 2 began to return to normal on the second day after challenge. However, the mean rectal temperature for pigs in treatment group 1 peaked lower and later at day 3 when compared to pigs in the other two treatment groups. These results would suggest that a possible benefit of vaccinating pigs at one day of age is a reduction in the febrile response to exposure to *S. choleraesuis* later in life.

A reduction in the febrile response may be an indication that the feed intakes of vaccinated pigs could be maintained or minimally affected in the face of exposure to *S. choleraesuis*. Thus, it is possible for weight gains to be maintained, reducing economic losses incurred when pigs become infected with *S. choleraesuis*. These results also suggest that the differences observed in clinical signs and rectal temperatures may have been associated in differences in feed intake. The mean day 0 weights of pigs in the three treatment groups were not significantly different when compared using a general linear model of least square means (p=.2080). The differences in mean day 14 body weights and mean weight gain per pig were determined to be statistically significant when pigs in treatment groups 1 and 2 were compared with pigs in treatment group 3 (Control). There was not a significant difference in average ending weight or average weight gain between treatment groups 1 and 2. Although there was no effort to measure feed consumption, the measured differences in

body weight gain suggest that there was probably better feed intake by pigs in treatment groups 1 and 2 when compared to pigs in treatment group 3. Dritz, et al. (1996) showed that reductions in weight gain occurred in pigs that had been injected with LPS. They postulated that weight loss was associated with reduced feed intake and partitioning of nutrients from consumed feed. These effects were also associated with increased levels of serum haptoglobin, a protein produced during the acute phase of an infection. Dritz, et al. (1996) concluded that the mechanism of this effect was due to a response to the cytokines that are produced during the stimulation of the immune response of the pig. *Salmonellae* are gram-negative bacteria, containing LPS in the structure of their outer membranes. Thus, a likely mechanism for the observed reduction in weight gain following infection by *S. choleraesuis* may include the production of cytokines, such as tumor necrosis factor-alpha (TNF-α), which reduce feed consumption and nutrient partitioning.

Subjective scoring of the pneumonia lesions from all pigs in this study indicated that IN vaccination also reduced the severity of pneumonia when compared with the pigs that were not vaccinated (treatment group 3). Fedorka-Cray, et al. (1995) demonstrated that the respiratory route might be important in the pathogenesis of infection by *S. typhimurium* following IN challenge. The original *S. choleraesuis* from which the vaccine strain was derived was isolated from a case of clinical salmonellosis in swine (Kramer et al. 1992). Therefore, SC54™ probably follows most of the pathogenesis mechanisms of its pathogenic parent strain. One of those mechanisms might include the invasion of pulmonary tissue either directly (Fedorka-Cray et al. 1995) or via infected macrophages (Kramer et al. 1992); (Roof et al. 1992), but without the development of the pneumonia typical of clinical swine

paratyphoid. Instead, a protective immune response is produced by residence of the vaccine strain in the pulmonary tissue. This immune response might be characterized by increased reduced lesions in the lung following infection and associated with increased IgA production or an increase in activated T-cells.

The mechanisms of protection from infection from *S. choleraesuis* are not fully understood. Perhaps an important feature of the protection afforded by SC54™ includes its presentation via macrophages and/or neutrophils (Roof et al. 1992) which have membrane-bound antigen to B- and T-cells in regional lymph nodes. Once such an immune response is stimulated, then perhaps the destruction of *S. choleraesuis* occurs more quickly than in vaccinated versus nonvaccinated pigs because of the presence of previously-primed immune cells.

Comparison of the bacteriologic examination results from the three treatment groups indicated that vaccination of pigs with SC54™ significantly reduces the populations of *S. choleraesuis* in the organs that were examined. In addition, it appeared that treatment group 1 pigs had significantly lower levels of *S. choleraesuis* isolated from the lungs when compared to treatment groups 2 and 3. There were no individual rectal swabs collected from any of the pigs from any of the three treatment groups. Therefore, it was not possible to evaluate what differences, if any, existed in the level or duration of fecal shedding of *S. choleraesuis* among treatment groups. It is reasonable to believe that the levels and duration of fecal shedding would have been lower in treatment groups 1 and 2, based on the responses that these groups displayed following challenge and comparison with previously reported effects of vaccination on fecal shedding (Roof and Doitchinoff 1995).

The mechanisms whereby pigs are able to shed *S. choleraesuis* for extended periods of time are not fully understood. It is apparent that the level of challenge dose of *S. choleraesuis* affects the magnitude of fecal shedding and the degree to which organs are infected with *S. choleraesuis* (Gray et al. 1996). Thus, pigs vaccinated with SC54™ may effectively reduce the challenge dose, thereby reducing the magnitude of fecal shedding and the degree to which organs are infected with *S. choleraesuis*.

Perhaps the ability of *S. choleraesuis* to survive for extended periods of time with in the hosts organs is one of the factors involved in fecal shedding for extended periods of time. These data suggest that the use of an avirulent, live *S. choleraesuis* vaccine blocks the development of organ colonization by virulent *S. choleraesuis*. This may be accomplished by competitive exclusion, stimulated T-cell populations or combinations of these and other immune response factors. Perhaps the effect of vaccination is to effectively decrease the challenge dose that is presented to the host animal since lower challenge doses are associated with lower levels of fecal shedding and lower levels of organ colonization. Previous challenge studies described an apparent association between tonsillar inoculation and the development of clinical signs and organ colonization (Reed et al. 1985; Roof and Doitchinoff 1995; Gray et al. 1996). Thus, it is postulated that the effective challenge dose may be reduced by a population of vaccine-activated T-cells within the tonsillar or intestinal tissue of pigs.

Vaccine study number one did not determine the duration of immunity afforded by vaccinating pigs at one day of age. Because pigs are frequently challenged at ages older than 35 days of age (Wilcock and Schwartz 1992), it would be important to know if the protection

achieved by vaccinating pigs at one day of age lasts as long as the protection afforded by vaccination at 21 days of age (Roof and Doitchinoff 1995). In addition to duration of immunity studies, the apparent benefits in weight gain should also be investigated to determine if the differences in weight gain reported in this dissertation are associated with more days to market weight. An additional finding from this experiment that should be further studied is the apparent improvement in clinical scores of suckling pigs seen immediately following vaccination. If the results reported here are repeatable, the potential use of this vaccine may also be to enhance farrowing crate performance of suckling pigs.

There were not any studies conducted to determine the effects of maternal antibody on the efficacy of the vaccine. It is not known what colostral antibody transfer occurred in these pigs and, therefore, what affect there may have been on vaccinated pigs. Therefore, vaccine efficacy should be compared between pigs with colostral antibody and those without colostral antibody to *S. choleraesuis*.

The results from vaccine study number 1 also indicated that piglets can be safely vaccinated at one day of age with a live attenuated *S. choleraesuis* vaccine (SC54™). Piglets that were vaccinated at one day of age were not adversely affected when the observed clinical signs were compared with the clinical signs of pigs that were not vaccinated. Vaccinated piglets appeared to have better clinical scores than did nonvaccinated piglets. It is doubtful that this difference is of practical significance because the difference in clinical scores was largely due to an increase in piglet mortality in the non-vaccinated group. Additionally, the analysis of the data showed a treatment-farrowing crate interaction which may have

confounded the results. It is concluded that there may be an additional benefit to the vaccination of neonatal swine.

Attempts to protect pigs against infection with *S. typhimurium* by vaccination with SC54™ at 21 d of age or at one day of age were not successful. In vaccine study number 2, the effect of vaccine was not evaluated against clinical challenge because of the failure the challenge dose to produce clinical disease in nonvaccinated pigs. However, it may be noteworthy to examine the differences in group mean daily rectal temperature. There was a response to the challenge dose of *S. typhimurium* by the pigs in this vaccine study, as evidenced by the elevation in mean rectal temperature by both treatment groups. Pigs from treatment group 2 (nonvaccinated) tended to have a higher mean rectal temperature when compared to pigs in treatment group 1 (vaccinated). When body weight gain was compared, treatment group 1 (vaccinated) pigs had significantly better weight gain during the course of the study. Vaccination was associated with fewer pig-days in which *S. typhimurium* was shed in the feces.

In vaccine study number 3, a severe clinical infection by *S. typhimurium* was induced by challenge of vaccinated and nonvaccinated pigs. Vaccination of pigs at one day of age with SC54™ failed to protect against clinical signs resulting from infection by *S. typhimurium*. It appeared that some of the pigs that were started in this study may have been infected with some other pathogen since the pre-challenge clinical signs of treatment group 2 were higher than the clinical scores of the other three treatment groups. When clinical scores for all of the treatment groups were compared for the days following challenge, only treatment group 1 had clinical signs that were near normal. The mean daily rectal

temperatures for each treatment group suggest also that some pathogenic mechanisms other than *S. typhimurium* infection were involved with some of the pigs. If so, such an infection may have altered the course of the experiment. Analysis of the results from bacteriologic examination of the organs from pigs of vaccine study number 3 indicated that pigs in treatment group number 2 were contaminated with *S. typhimurium*. Treatment group number 1 was to have served as nonvaccinated, uninoculated controls and treatment group 2 was to have served as the vaccinated, non challenged control group. There was apparently some cross-contamination of *S. typhimurium* between the two rooms that housed the different groups of pigs. *Salmonella typhimurium* may have been brought into the groups via the feed. However, there were no feed samples collected and tested for the presence of *Salmonella*. Another possible source of contamination could have been from animal caretakers since each pig in each room was handled daily in order for rectal temperatures to be taken. A third potential source of infection was the pigs themselves. Bacteriologic screening of the source herd from which these pigs were obtained later showed that all fecal samples collected from the floors of pens in a nursery were positive for *Salmonella* serogroup B (D. Baum, unpublished data, 1997).

In vaccine study number 4, it was shown that the vaccination of pigs at about 56 days of age (two weeks after placement into finishing facilities at 7 weeks of age), significantly reduces the levels of *Salmonella* detected in mesenteric lymph nodes of pigs at slaughter. Analysis of the populations of *Salmonella* that appeared to be affected indicate that *Salmonella* serogroups B and C1 are significantly reduced by vaccination while levels of serogroups C2 and E were higher in vaccinated groups of pigs when compared to

nonvaccinated groups of pigs. Thus, vaccination of pigs with SC54 ™ appears to be an effective management procedure for reducing the numbers of pigs that carry certain serogroups of *Salmonella*. Further studies should be conducted to determine if vaccination of pigs at one day of age results in the same reduction of mesenteric lymph node culture-positive pigs. An experimental study should also be conducted to determine if there is any cross-protection afforded to pigs that have been challenged with members of *Salmonella* serogroup C2.

When individual serotypes are studied for differences in reduction between the two treatment groups, vaccination significantly reduced the levels of *S. derby, S.* 4:12;i monophasic, *S. heidelberg, S. choleraesuis, S. hartford*, and *S. braenderup. Salmonella typhimurium* was not significantly reduced, which is consistent with results from vaccine studies 2 and 3. The mechanism of protection against infection by *S. typhimurium* has been suggested to be related at least in part to the O-side chains of the LPS of the organism in pigs (Lumsden and Wilkie 1992) and in chickens (Hassan and Curtiss 1994). In the study by Lumsden, protection against infection by *S. typhimurium* was shown to be mediated by the side chains of the LPS of *S. typhimurium*. In the study by Hassan and Curtiss (1994), chickens were vaccinated with a live avirulent *S. typhimurium* and challenged with representatives from serogroups B, C, D, and E. Protection was afforded to chickens when they were challenged with representatives of serogroup B, some protection to challenge from serogroups D and E and less protection to challenge by representatives from serogroup C. Therefore, there appears to be other antigens in addition to LPS that are involved with the development of immunity to *Salmonella* infections.

These findings do not suggest that vaccination cause infections by serogroups C2 and E. It is postulated that there were more vaccinated pigs that were exposed to serogroup E than there were nonvaccinated pigs and that vaccination had no effect in reducing the number of culture-positive (serogroup E) pigs. It was somewhat surprising to find that vaccination did not reduce the isolation of *Salmonella* from serogroup C2. Serogroups C1 and C2 share a common O-antigen (O:6). If protective immunity is directed against portions of the outer membrane of *Salmonella*, including LPS (as suggested by Hassan and Curtiss, 1994), it would be reasonable to believe that there would be some cross-protection against infection by C2 *Salmonella* by a C1 *Salmonella* vaccine. Experimental challenge studies using the C2 isolates that were recovered from the mesenteric lymph nodes should be conducted to determine if vaccine protects against infection by these isolates. It would be important to know what effect vaccination would have on pigs challenged with isolates from these two serogroups.

It is unknown if the timing of vaccination in relationship to the expected exposure would have any effect on the outcome of culture prevalence at slaughter. For instance, a repeated study of the same design could be conducted in which pigs were vaccinated at one day of age, 21 days of age, and 42-56 days of age. Also, since it has been shown that *S. typhimurium* can be detected in mesenteric lymph nodes only 6 hours after intranasal challenge (Fedorka-Cray et al. 1995), it might be possible for pigs to become infected if they are in close proximity to pigs that are actively shedding *S. typhimurium* via aerosol. However, since all pigs sampled from each treatment group in this study were not exposed to pigs from the other treatment group by direct nose-to-nose-contact and since there was never

more than 3 hours spent by any of the pigs in lairage, it seems unlikely that cross-contamination between or within treatment groups occurred immediately prior to slaughter. An experiment should be conducted in which pigs that have been vaccinated at one day of age are challenged with *S. typhimurium* as previously described (Fedorka-Cray et al. 1995). Such an experiment would determine if vaccinated pigs have reduced organ colonization immediately after receiving an infective dose of *S. typhimurium*. It would also be useful to evaluate the effectiveness of vaccinating pigs near slaughter with SC54™. Such an experiment might determine if vaccination could be used as an intervention strategy in herds that are found to be positive at slaughter. If reduced organ colonization could be demonstrated in either of these experiments, then SC54™ might be shown to have further applications in food safety programs directed toward reducing *Salmonella*.

In addition, the results from vaccine study 4 indicate that vaccination may reduce the magnitude of the serologic response of pigs to exposure to *Salmonella* and reduce the seroprevalence of *Salmonella* in groups of pigs. It was shown that the mean mix-ELISA OD% of vaccinated animals was significantly lower in vaccinated animals when compared to nonvaccinated animals. This would suggest that the magnitude of antibody response to challenge by *Salmonella* is lower in animals that have been vaccinated. It was also shown that there was a significant reduction in the seroprevalence of *Salmonella* when vaccinated pigs were compared with nonvaccinated pigs. This difference was observed when a mix-ELISA OD% cut-off of 40 was used to determine whether or not a serologic response was positive or negative. However, when each group of pigs was categorized according to the Danish method of categorization (Nielsen and Bager 1995), there was no difference in the

distribution of herds among levels 1, 2, and 3. When the OD% cut-off was increased to 100, a difference in the distribution of the treatment groups based on seroprevalence was noted. Groups of pigs that were vaccinated had a higher percentage of level 1 groups of pigs and a lower percentage of level 3 groups of pigs. These results may have reflected a problem that was noted regarding the quality of the meat juice samples that were collected at slaughter. Since most of the meat juice samples were cloudy and necessitated being centrifuged prior to analysis. This problem with sample quality may have been due to the method of sample collection. In Denmark, special meat collection tubes have been used for sample collection and were used for some portions of the epidemiologic studies of this dissertation. However, only sterile plastic bags were used for muscle sample collection for the purposes of this portion of the study. This sort of contamination may have caused nonspecific reactions to occur during the analyses since the method used to assay antibody relies on the percent of light that passes through the tested sample. Perhaps the enzyme conjugate was allowed to bind nonspecifically to the microtiter plate wells.

In addition to the effects of vaccination on the magnitude of the serologic response to *Salmonella* and the seroprevalence of *Salmonella*, the effect of culture status on these parameters was also investigated. It was shown from these studies that there was a significantly lower mean OD% in pigs that were culture-negative when compared to pigs that were culture-positive for the presence of *Salmonella*. It was also shown that culture-negative pigs had a lower seroprevalence of *Salmonella* antibodies when compared to culture-positive pigs. These results indicate that the Danish mix-ELISA would be useful to monitor groups of pigs in the United States for the presence of *Salmonella* in this one pig production system,

using an OD% cut-off of 40. This tool would also be likely to be useful in the swine herds of the United States for monitoring for the presence of *Salmonella* since the serotypes of *Salmonella* found throughout the United States (Ferris and Miller, 1996) tend to the same serotypes as were found in this one herd (*S. typhimurium, S. choleraesuis, S. heidelberg, S. derby,* and *S. infantis*).

These results suggest that SC54™ use is associated with reduced exposure via reduced shedding of *Salmonella*.

In vaccine study number 5, there was no difference between the two treatment groups that were tested for the presence of *Salmonella* antibody with the mix-ELISA. Another study of this kind should be conducted to confirm whether or not pigs vaccinated with SC54™ have detectable antibody at slaughter. This would be important information in the event that SC54™ is used as an intervention strategy in a *Salmonella* reduction program. If it can be shown that there is no detectable antibody in pigs that are vaccinated at 1, 21, or 42 days of age with SC54™, then the vaccine's use in such a control program would be of great value in that the vaccine could be used to reduce levels of *Salmonella* without causing an increase in the seroprevalence of *Salmonella*. This presumes that serologic monitoring of herds would be used to assess herd *Salmonella* levels.

Epidemiologic studies

Eight different serotypes of *Salmonella* were isolated from feces collected from the floors of pens from Farms A, B, and C. Six of these serotypes have been listed by the CDC as the most frequently isolated serotypes from human and non-human sources (Bean and Potter 1992), further demonstrating that there are organisms of zoonotic importance present

on some swine farms in the United States. Results similar to these have been reported elsewhere (Galton et al. 1954; Greenberg et al. 1963; Kampelmacher et al. 1963; Hansen et al. 1964; Lee et al. 1972; Hartwig and Jones 1976; Childers et al. 1977; Garcia et al. 1978; McKinley et al. 1980; Currier et al. 1986; Tay et al. 1989; Kramer et al. 1995; Fedorka-Cray et al 1996). Thus, food safety programs directed at reducing the levels of *Salmonella* in the slaughter plant should include some method of monitoring and reducing *Salmonella* levels on the swine production farm. Such programs should include some method of being able to determine whether there is an association between the reduction of *Salmonella* on the farm and the reduction of *Salmonella* on pork and pork products in the slaughter plant.

There were differences in culture prevalence noted in samples collected from Farms A, B, and C. Both Farms B and C had had history of infection by *S. choleraesuis* in the year prior to the beginning of this study and Farm B had instituted a vaccination program in the year prior to this study. It must also be stated that the sampling procedures for the three farms were different. At each visit to farms A and B, all pens in the building were sampled; random samples from all pens in each finishing site of Farm C. This sampling method may have resulted in a higher observed prevalence of *Salmonella* for Farms A and B. However, the sample prevalence from Farms A and B were considerably lower than the sample prevalence of Farm C. When the group prevalence from each farm is compared, Farms A and B also had fewer groups of pigs from which *Salmonella* was isolated. This suggests that farms with higher group culture prevalence of *Salmonella* may have a higher sample culture prevalence of *Salmonella*. These findings would suggest that bacteriologic culture methods are adequate for determining the culture status of groups of pigs but perhaps not for

individual pigs. Disadvantages of bacteriologic examination would include the possibility of high false-negative results reported (low sensitivity), the time needed for determination of *Salmonella*-positive (approximately 5-7 days of laboratory time), and high test costs ($15.00 - $25.00, depending on laboratory and serotype costs). In contrast, ELISA testing needs less time (1-2 days) and costs less ($6.00 per sample). Therefore, routine bacteriologic testing of swine and swine farms may not be conducive for use as a rapid, economical *Salmonella* screening program for swine herds. However, culture would be invaluable for investigations of farms that have been shown to have high levels of *Salmonella*. At issue, then is what method(s) should be considered for use as a rapid, economical screening procedure for use in a *Salmonella* monitoring program.

Results from the analysis of the serum samples that were collected at the same time as environmental pen fecal samples were collected suggest that the mix-ELISA would be adequate for the rapid and economical monitoring of swine farms for the presence of *Salmonella*. When the serologic and bacteriologic results from the three farms are compared (Tables 38, 39, and 40) the usefulness of the mix-ELISA is apparent. When all of the groups of pigs from Farms A, B, and C are assigned a *Salmonella* level according the system used in Denmark (Nielsen et al. 1995), the two farms with the lowest culture prevalence of *Salmonella* also have the highest number of *Salmonella* level 1 groups of pigs. Farm C, with many groups of pigs that were culture-positive for the presence of *Salmonella* also had the highest number of level 3 groups of pigs.

Although the seroprevalence difference between culture-positive groups of pigs and culture-negative was not statistically significant (Table 41, p =.0872), there did appear to be a

trend in the number of culture-positive groups of pigs that were classified into *Salmonella* level 1 and 3 (Table 41). There were 21/29 (72%) level 1 groups of pigs that had a *Salmonella* culture prevalence less than 1%. This compares to 7/16 (44%) of level 3 groups of pigs that had a *Salmonella* culture prevalence of less than or equal to 1%. Level 1 groups of pigs had fewer groups of pigs that had *Salmonella* culture prevalence greater than 1% (8/29 groups or 28%). Level 3 groups of pigs have a higher proportion of groups that had a *Salmonella* culture prevalence of greater than 1% (9/16 groups or 56%) and a lower proportion of groups of pigs with 1% or less culture prevalence. These results agree with those that have been reported from farm investigations of *Salmonella* prevalence in Denmark (Nielsen et al. 1995; Nielsen et al. 1996) in which it was shown that the mix-ELISA results correspond with the culture prevalence results from farms over time. In these two studies, it is important to note that a few herds that have been determined to be *Salmonella*-negative by culture had a moderate proportion (between 10% and 15% seroprevalence) of sero-positive animals. One possible explanation for this phenomenon is that the results reported from the mix-ELISA were actually false-positive results. This would be unlikely in light of the specificity and sensitivity reported for the mix-ELISA (90% and 96%, respectively, Nielsen, personal communication). Another likely explanation for these results is that culture methods may not have been sensitive enough to detect low levels of *Salmonella* on these farms. The Danish herds were determined to be negative by the absence of clinical signs and failure to culture *Salmonella* from pen fecal samples or rectal swabs collected from pigs on the farms. However, as noted in the original paper reporting the mix-ELISA (Nielsen, et al 1995), there are animals that that may have an elevated serologic response in the mix-ELISA,

have no detectable *Salmonella* in environmental pen feces, yet harbor *Salmonella* in mesenteric lymph nodes. Finally, it has been shown that swine can clear an infection by *Salmonella* yet remain seropositive (Gray et al, 1996).

Without a complete year of culture results from Farm C, it is difficult to assess the association of culture and season of the year. However, the data set from Farms A and B suggest that there could be a seasonal affect associated with the presence of *Salmonella* as detected by culture methods. This is in contrast to the finding of no seasonal variation in *Salmonella* culture prevalence of Dutch pigs (Van Schie et al 1987)

These data have been analyzed by attempting to correlate farm culture results with farm serologic results and by evaluating the correlation coefficient between individual bacteriologic and culture samples (Baum et al. 1996). Those results indicated that there was no significant correlation coefficient calculated for group culture and serologic results. Failure to demonstrate a correlation between culture and serologic prevalence of *Salmonella* infections has been reported elsewhere (Nicholas and Cullen 1991). Further, this method (correlation coefficient calculation) of comparing serologic and bacteriologic data from a farm is not preferred to simple descriptive statistics (Thrusfield 1995). Thus, other methods of demonstrating associations between culture prevalence and serologic prevalence must be used to compare these methodologies. When one considers the dynamics of *Salmonella* shedding and the immune response that accompanies infection with *S. choleraesuis* or *S. typhimurium*, (Nielsen et al. 1995; Gray et al. 1996) for instance, detectable levels of *Salmonella* in the pen feces are found largely within the first 10-14 days after challenge. This coincides with the time that peak IgG levels begin to appear in the serum. The rate of

decline for serum IgG is slower than the reduction of the shedding of organisms in feces. Therefore, it would be expected that animals with high levels of serum antibody might indeed be culture-negative when rectal swabs and pen fecal samples are examined.

There are two advantages of using the mix-ELISA instead of culture for the routine monitoring of swine farms for the presence of *Salmonella* include rapid turn-around time for results (1 day laboratory time), and lower cost per test (approx. $6.00 per sample). Disadvantages of using the mix-ELISA would include an inability to identify the serotypes involved with the immune response, and the inability to detect *Salmonella* from serogroup E or other serogroups that would not posses O antigens 1, 5, 6, 7, or 12.

The results from bacteriologic and serologic studies conducted on samples collected at slaughter from pigs from Farms A, B, and C further indicate that the mix-ELISA may be useful in the United States for monitoring swine herds for the presence of *Salmonella* and that the use of meat juice instead of serum may be an additional advantage since routine sampling could be conducted within a slaughter plant rather than being conducted on a swine farm. Meat juice has been reported to be an adequate medium to test for the presence of circulating antibody to *Salmonella* (Nielsen et al. 1996) with a specificity and sensitivity of 0.95 -0.99 and 0.81-0.89, respectively. The point of testing for the presence of antibody at slaughter is not to have a method for sorting pork carcasses that are positive for *Salmonella* antibody but to use information collected at slaughter to provide to producers and health advisors for intervention in subsequent groups of pigs for the purpose of reducing the levels of *Salmonella*.

Although there were not enough sample points for Farms A and B to make definitive conclusions, there were some trends worthy of mention. For instance, groups of pigs from Farm A that were slaughtered in the months of August, and November were classified as level 1 groups of pigs as determined from the mix-ELISA results from meat juice samples collected at those times. During those months, though, there were positive culture results from lymph nodes collected at slaughter. *Salmonella enteritidis* (see Table 26) had been demonstrated in previous samples of finishing pens. *Salmonella anatum* had not been demonstrated to be present on this farm in the previous year. One explanation for these results is that the *S. enteritidis* was carried into the slaughter facility from the farm and perhaps the *S. anatum* was ingested by pigs during lairage. If this were the case, then the role of mixing pigs of different *Salmonella* status in lairage is an important consideration in a *Salmonella* reduction program. One of the reasons for the reduction in *Salmonella* in fresh pork in Denmark following their reduction program is the ability of slaughterhouses to identify herds with high levels (level 3) of *Salmonella*. Pigs from level 3 farms are slaughtered separately from level 1 and 2 pigs. The pork from level 3 farms is not permitted to enter the fresh pork market (Nielsen et al. 1995). A study should be conducted to determine if it is possible to slaughter level 1 pigs in a commercial slaughter facility in the United States, observing segregation in lairage, to determine if segregation of pigs would reduce the culture prevalence of *Salmonella* on pork products.

The results from the mesenteric lymph node data from Farm B also indicate that serology of meat juice at slaughter is an indication of the *Salmonella* status of the farm of origin. All of the groups of pigs that were tested on the farm were classified as *Salmonella*

level 1. However, there was one group of pigs that was slaughtered that had a seroprevalence greater than 15% and were classified as level 3 (Aug-95 group, Table 49). This change in *Salmonella* level also coincides with the isolation of *Salmonella* from the mesenteric lymph nodes during the same month and in the month that followed. *Salmonella agona* had been isolated from previously from this farm.

As mentioned above, it seems likely that there are two different populations of *Salmonella* that were present in the mesenteric lymph nodes of the swine sampled from Farms A and B. One population would represent the population from their farm of origin while another population might represent serotypes that were picked up while in after leaving the farm but before slaughter. Since it has been demonstrated that it is possible for mesenteric lymph nodes to become infected with *Salmonella* shortly after ingestion or intranasal exposure (Reed et al. 1985; Fedorka-Cray et al. 1995), it is possible that the "new" serotypes for these two farms were actually picked up from the environments that these pigs were in prior to slaughter. The pigs from Farm A were commingled with pigs from other farms at a central collection point, held for 1-2 h and hauled for 3 h to the slaughter facility where they were held for an additional 1-2 hours prior to slaughter (J. Ryan, personal communication). Additionally, the trailer in which the pigs transported may have been contaminated. Contaminated transportation equipment has been associated with increased culture prevalence of *Salmonella* (Berends et al, 1996)

Pigs from Farm B were shipped directly to slaughter for the sampling times of Jun-95 and Jul-95 and then were sent by common carrier for the sampling times of Aug-95 and Sep-95 (D. Fisher, personal communication). In addition Farm B pigs were held overnight for the

Sep-95 sampling due to a mechanical problem at the slaughter plant that resulted in a cessation of operations for a day. At the beginning of 1996, *S. choleraesuis* infections were diagnosed in this farm (D. Fisher, personal communication). This finding was of interest in light of the culture findings at slaughter and on the farm: culture findings at slaughter can indicate the culture status of the farm of origin.

When the different groups of pigs from Farms A, B, and C are categorized by *Salmonella* level and culture status, most of the groups of pigs are either level 1 or 3 and had over 1% culture prevalence of *Salmonella*. These results suggested that the pigs that were tested were recently infected but not able to develop a measurable antibody response prior to slaughter. Thus, regular serologic monitoring of pigs at slaughter would be useful to determine what groups of pigs originate from farms with high levels of *Salmonella*.

These results seem to be in disagreement with the results obtained from the farms. However, it is important to note that the farm samples were collected 2-4 week prior to slaughter. It is possible, then, that infections of these pigs by *Salmonella* occurred at about the time that farm samples were collected or some time afterwards. This implies that those responsible for sample collection on the farms actually brought the organisms to the farm! More likely, though, these findings suggest that there may have been some breakdown in biosecurity on these farms just prior to the time of sales such as the introduction of organisms from farm workers who load pigs (Berends et al 1996)

Mesenteric lymph nodes and meat samples were collected at slaughter from pigs from a fourth farm, Farm D. This farm had a history of low seroprevalence from data collected for use in the serologic survey of farms for this dissertation. When the results from this farm

were tabulated (Table 53), it appeared that *Salmonella* level 1 groups of pigs had more culture-negative samples compared to *Salmonella* level 3 groups of pigs.

These results suggest that ongoing serologic monitoring of groups of pigs is necessary to determine the *Salmonella* status of the farms that produce the pigs which might explain the difference in the two different studies. The first epidemiologic study was conducted for almost an entire year for most of the farms, while the period of time in which samples were collected for epidemiologic study number 2 was much shorter and confounded with mechanical difficulties in the collection of samples from Farms A and B.

The bacteriologic and serologic data indicate that the serologic monitoring of swine herds is an effective method of determining whether or not a herd has a high prevalence of *Salmonella*. The differences in test costs also favor the use of serology to monitor herds for the presence of *Salmonella*.

After examination of the serologic and cultural data from samples collected from Farms A, B, C, and D, it appeared that there might have been a seasonal trend in the seroprevalence of *Salmonella* as well as a possible association between the use of an isolated nursery and lower *Salmonella* seroprevalence.

Results from examination of all of the serum samples collected from the 267 groups of pigs indicated that there was a significant difference in the seroprevalence of *Salmonella* antibodies and in the magnitude of the serologic response (mean OD%) when quarter of year and the use of isolated nurseries were compared. The overall sample seroprevalence of *Salmonella* antibodies from this database of herds was 27.5%. These results suggest that there should have been a difference in the *Salmonella* seroprevalence when season of

placement into the finishing buildings was evaluated. When the questionnaire data were analyzed, a difference in seroprevalence was associated with the season of placement into finishing buildings. These results support the suspicion from the culture data that there were seasonal variations in *Salmonella* prevalence.

These results support work (Dahl et al. 1996) that has been reported regarding the on-farm reduction of *Salmonella* by the "strategic removal of pigs from infected herds". It can be successfully argued that the premise for the use of an isolated nursery is a strategic removal of pigs from an infected herd. This report describes the value of removing pigs from an infected herd. The serologic data reported in this dissertation support the contention that there is lower exposure to *Salmonella* when an isolated nursery (strategic removal of pigs from the farrowing farm) is used as part of the farm's pig management strategies.

The results of the analysis of the questionnaire indicate that there are 14 areas within the scope of pig management that should be evaluated for control of the seroprevalence of *Salmonella* in swine herds. This conclusion is made because of the odds likelihood of the following factors that were found to be associated with higher seroprevalence of *Salmonella*: audit score greater than or equal to 7, failure to remove dead pigs daily from the finishing building, feed manufactured on the farm, dry feed system compared to a wet/dry feed system, pelletted feed being fed, more than one day used to fill a finishing complex, practicing continuous flow pig management, having production located principally on two sites (not to be confused with two-site production systems described elsewhere (Alexander and Harris 1992), no isolated nursery, use of recycled flush water for manure removal, more than one nursery source used to fill a finishing building or complex, no nozzle on boot hose in

finishing buildings, finisher filled in the second quarter of the year (April-June), and transport personnel allowed in the finishing building. These compare to the risk factors identified in a review by Berends et al (1996): lack of farm hygiene, lack of transport hygiene, and a *Salmonella*-positive source farm. In contrast, the work reported in this dissertation specify what areas of hygiene should be addressed.

It was demonstrated by the data collected from the questionnaire that there is a tendency to have higher seroprevalence of *Salmonella* at the end of the finishing period if the placement audit score (audits) was greater than or equal to 7. A method of auditing groups of finishing pigs by management may have been developed by virtue of the use of this questionnaire. With the given management practices that were shown to be associated with the development of antibodies to *Salmonella*, it is likely that the regular monitoring of the parameters included in the questionnaire may aid in the reduction of risk to *Salmonella* in groups of pigs in the finishing building. It would be of benefit to be able to continue the collection of questionnaire information, to expand on the data points available, and to continue the analysis of these risk factors.

The daily removal of dead pigs is also important for the control of *Salmonella* exposure in a finishing building. These results are in agreement with those of Henken et al. (1992) who also found that daily dead animal removal was important for reducing the risk of developing *Salmonella*. Although it has been demonstrated that *S. choleraesuis* can be destroyed by the composting of pig carcasses (J. Garcia-Sirera et al. 1996), the likelihood of spread of *Salmonella*, including *S. choleraesuis* appears to be greater if the dead pig carcasses

are not removed daily. The conditions of composting are obviously different from the conditions within a decaying pig carcass located in a pig building.

As was reported in a survey of *Salmonella* in feed (Harris et al. 1997), there appeared to be a possible association between the finding of *Salmonella* on the farm with the use of farm personnel in manufacturing feed. These results suggest that the on-farm methods of manufacturing and/or storage of ingredients used in manufacturing of swine feed may put the farm at risk for the development of *Salmonella* infections. The results reported from the survey conducted in this dissertation also suggest that farms that utilize on-farm feed manufacturing equipment may have a higher likelihood of developing increased seroprevalence of *Salmonella*.

The association of dry feed with an increase in *Salmonella* seroprevalence might reflect poorer feeder sanitation or the introduction of serotypes through the feed. Indeed, it has been suggested that the use of acidified feed or water may reduce the levels of *Salmonella* in some herds (Dahl et al. 1996). Perhaps the microenvironment of pig feeds tend to be pH neutral or pH basic. The pH, then might be varied by the addition of water in some wet/dry feeding systems that allow pigs to mix water with dried feed. It is also curious that pelleted feeds were associated with higher prevalence of *Salmonella*. There are at least two possible explanations for this. First, the preponderance of *Salmonella*-positive groups of pigs came from one farm that manufactured its own pelleted feed. Therefore, the data may have been skewed to obtain this result. Another explanation is that the pelletting procedure used by this farm may not have an adequately hot pelletting mill to destroy feed that could have been contaminated with *Salmonella*. A third explanation is that the pelletting

procedures used by the farm allowed for the post-pelleting contamination of the completed pig feeds.

Four other factors associated with increase seroprevalence of *Salmonella* were associated with pig flow management: more than one nursery source used to fill a finishing complex, more than one day taken to fill a complex, continuous flow pig management, and some form of production system in which facilities are located principally on two sites. It is essential that these three areas be carefully adhered to for the procuring and rearing pigs of improved health status (Alexander and Harris 1992). The successful all-in-all-out system requires careful attention to farrowing schedules so that when pigs are weaned, they are weaned within a very narrow time period which would be defined by their average age and the standard deviation of that age. The association of no isolated nursery and high *Salmonella* seroprevalence should cause a swine unit manager to give pause to the value of isolated nurseries.

The failure to use a hose with a nozzle being associated with the seroprevalence of *Salmonella* suggests that in farms where a hose with an attached nozzle is available, that it might be used more frequently or effectively than when no attached nozzle is available. All farms reported some sort of hose used in the finishing building for boot sanitation. Therefore, it is possible that the addition of a nozzle to the end of a hose helps with compliance of personnel in the cleaning of boots between buildings. The physical spread of *Salmonella* among pens in pig finishing buildings can be reduced by simple sanitation and labor traffic patterns (Dahl et al. 1996).

The association of increased *Salmonella* seroprevalence in pigs that were placed in their finishing buildings during the period of April through June should also give swine unit managers pause to oversee compliance of biosecurity measures especially during that time of year. This might be the time when the addition of organic acids, extra attention to sanitation of facilities and personnel and the use of SC54™ vaccine might be needed in herds that are at risk. Certainly, those herds that do not have an isolated nursery should carefully review their management practices during this time of year. Continued monitoring of swine herds is recognized as important to the reduction of *Salmonella* infections in swine in Denmark (Nielsen et al. 1995; Nielsen et al. 1995; Mousing et al. 1996; Nielsen et al. 1996).

The presence of personnel associated with loading swine at the time of marketing was also a risk factor identified to place a finishing complex at risk for the development of increased *Salmonella* seroprevalence.

Areas within a swine unit that must receive attention in the efforts to reduce *Salmonella* deal largely with what could be called sanitation procedures, as outline above and described elsewhere (Baggesen et al. 1996; Dahl et al. 1996; Dahl et al. 1996). Critical control points that must be considered in *Salmonella* reduction programs all involve adherence to sanitation procedures and should include the consideration of modifying facilities to incorporate isolated nursery facilities.

The reason for ongoing monitoring of farm management practices and assessment of risk factors associated with the development of seropositive groups of pigs can be strictly economical. The data obtained from the few farms that submitted information about pig weight gain suggest that there is a large benefit in terms of performance in the finishing

building if groups of pigs are not exposed to high levels of *Salmonella*. The difference between *Salmonella* level 1 and *Salmonella* level 3 groups of pigs is about 5 pounds per square foot of building per year. Currently, finishing buildings used in the United States are about 8,000 sq. feet in area. An additional 5 pounds of live pig produced per square foot of building would be an additional 40,000 pounds of live pig per year. If marginal income per pound of live pig is $0.15, then the potential exists for an additional total marginal income of $6,000 per 8,000 square feet of building. These results do not conclude that *Salmonella* seroprevalence is the only contributing factor to reduced performance in a finishing building. However, the monitoring of *Salmonella* seroprevalence may be useful as an indicator of the level of management for a finishing building. Again, continued collection of data would be useful to be able to determine if these differences in performance are found throughout the United State's swine production systems.

The results from the serologic evaluation for *Toxoplasma* and Trichina suggest that the seroprevalence of these two pathogens in confined swine herds in the United States is almost 0. The few positives that were reported could easily have been false-positives. This would be expected in the instance of a disease with very low prevalence (Thrusfield 1996). Furthermore, one farm from which a positive serologic response to Trichina ELISA was obtained also tests all of its swine carcasses for the presence of Trichina. The test used is a digestion procedure. To date, there have been no positive results detected from the use of the digestion procedure for testing of Trichina. It might be that these two zoonotic agents are of such low prevalence in modern swine herds to be considered eliminated or near elimination from such swine herds.

The presence of *Y. enterocolitica* O:3 antibodies in some groups of pigs is of interest. As reported in other studies of the distribution of antibodies to this pathogen (Wingstrand and Nielsen 1996), herds seem to be categorized into one of three seroprevalence levels: low, medium, and high. What is interesting from data presented in this dissertation is the apparent association between the sample and group prevalence of *Salmonella* and *Y. enterocolitica* O:3 antibodies. It would seem, then, that in units where there is good sanitation and reduced levels of *Salmonella*, there might also be reduced levels of *Y. enterocolitica* O:3. As postulated earlier (Wingstrand and Nielsen 1996), attention should be paid to controlling the pig-to-pig fecal contact that might contribute to the spread of *Y. enterocolitica* O:3 within a herd. The most frequently isolated serotype of *Y. enterocolitica* in the United States is O:8 (Kotula and Sharar 1993). However, isolations of O:3 and O:5 have been made from swine or pork products from swine produced in the United States. Further prevalence studies would be necessary to determine the frequency and distribution of these serotypes in the United States' swine population and whether or not an association exists between *Salmonella* prevalence and *Y. enterocolitica* O:3 and O:5 prevalence. If the association does exist, then might be possible to determine if the same risk factors identified for *Salmonella* seroprevalence also are associated with *Y. enterocolitica* seroprevalence. Thus, changes made in farm management practices to affect the reduction of *Salmonella* seroprevalence might reduce the *Y. enterocolitica* seroprevalence.

In light of the FSIS' Pathogen Reduction; Hazard Analysis Critical Control Point (HACCP) Systems; Final Rule (mega-reg) (USDA 1996) this is valuable information for the pork industry. The results from the cross-protective abilities of SC54™, the serologic

response of pigs vaccinated with SC54™, the reduction of *Salmonella* seroprevalence by the use of SC54™, the development a procedure to audit farm management practices, and the association of improved growth performance of pigs with reduced levels of exposure to *Salmonella* can have immediate application to on-farm pathogen reduction and production management.

For instance, in a *Salmonella* reduction program, enacted by the mega-reg, it is likely that some method will be needed to identify swine farms that produce pigs with high levels of *Salmonella*. I propose that routine serologic monitoring of meat juice from pork muscle collected at slaughter be conducted as is done in Denmark, using the mix-ELISA. Intervention visits by slaughter house personnel and swine veterinarians would be made to swine farms with high seroprevalence of *Salmonella*. At the time of these intervention visits, swine veterinarians would conduct an audit of farm management practices and collect samples of feces for bacterial culture. Analysis of the audit data and serotypes isolated would assist in determining what changes should be made in farm management practices. Knowledge of the serotypes of *Salmonella* involved with an increase in seroprevalence would help to determine if vaccination with SC54™ was warranted. For instance, if *S. typhimurium* were found on a farm, vaccine may not be recommended for use to reduce prevalence. Rather, it would need to be impressed upon the management of the farm that strict adherence to good management practices such as proper cleaning, sanitation and pig flow will reduce the levels of *Salmonella* (Baggesen et al. 1996; Dahl et al. 1996; Dahl et al. 1996). On the other hand, should culture results from the farm lead to the discovery of serotypes that appear to be reduced by SC54™, then vaccination could be used as an adjunct to the above-

mentioned management practices. Vaccination of pigs with SC54™ would also expedite the reduction of *Salmonella* seroprevalence, which assist the producer in being able return to selling pigs into the low prevalence market. The use of SC54™ in a *Salmonella* control program would not interfere with the routine serologic testing of meat juice samples from a vaccinated herd since it was demonstrated that pigs vaccinated with SC54™ do not produce a level of antibody detectable by the mix-ELISA.

The routine auditing of the management of groups of pigs and the collection of growth and feed information will allow producers and swine veterinarians to monitor improvements in the biological performance of pigs within a farm. Auditing of groups of pigs at the time of placement into the finishing building would also help to identify groups of pigs that would be at risk for becoming exposed to *Salmonella*. This information would be useful in determining what preventative measures should be considered for that group of pigs. Such changes might include restricting human traffic into and within the finishing building, addition of nozzles to the ends of boot hoses in the finishing building, and whether or not to consider medication or vaccination of the group of pigs.

The data created by such a monitoring system would need to be processed by a central information system that would collate serologic, bacteriologic culture, and farm audit information. Regular reporting of results to pork producers would be made from this information system as part of an integrated system for continuous improvement of the quality of pork produced in the United States.

CHAPTER SIX. CONCLUSIONS

The results from the vaccine studies indicate that one-day old pigs can be safely vaccinated against *S. choleraesuis* infections with SC54 ™. Pigs that were vaccinate at one day of age had fewer organs that were colonized by *S. choleraesuis* when compared to pigs vaccinated at 21 days of age and pigs that were not vaccinated. Pigs that were vaccinated at one day of age had normal clinical signs and mean rectal temperatures for a longer period after challenge than did the pigs that were vaccinated at 21 days of age and those pigs that were not vaccinated.

It was also shown that the vaccination of pigs after they had been moved to finishing buildings reduces the prevalence of *Salmonella*. The culture prevalence of serogroups B and C1 was significantly reduced when vaccinated pigs were compare to nonvaccinated pigs. The serologic prevalence was significantly reduced when vaccinated pigs were compared to nonvaccinated pigs. Therefore, the use of SC54 ™ is an intervention to reduce the prevalence of *Salmonella* in finishing buildings.

The results from the epidemiologic studies showed that the mix-ELISA can be used in the United States to detect groups of pigs that are infected with *Salmonella*. It was shown that seroprevalence is associated with culture prevalence. These associations were demonstrated when the culture prevalence of *Salmonella* isolated from feces samples were compared with the serologic prevalence of pigs when samples were collected from farms. The culture prevalence of *Salmonella* from mesenteric lymph nodes was also associated with the serologic prevalence of *Salmonella* antibody in muscle juice collected at slaughter.

There also appeared to be an direct association between the farm seroprevalence of Y. enterocolitica O:3 and the farm seroprevalence of *Salmonella*.

There was a very low seroprevalence (less than 0.1%) of *Toxoplasma gondii* and *Trichinella spiralis* antibody.

The results from the epidemiologic studies also identified management practices that were associated with increased seroprevalence of *Salmonella* in pigs at or near slaughter. Groups of pigs with *Salmonella* seroprevalence greater than 15% had poorer growth performance than groups of pigs with *Salmonella* seroprevalence less than 10%.

APPENDIX

The following data set contains the data that were used to compare the information collected from the audit data sheet (Table 2).

Following are the definitions of the headings of each column.

FARM CODE:	As explained in the materials and methods section of this dissertation.
DATE SPLED:	The date that samples were collected.
MONTH SPLED:	Number of the month in which samples were collected.
QTR SPLED:	Qtr of the year in which samples were collected.
NO. SPLES:	Number of samples collected
AVG. OD.:	Average OD for samples collected
MIN. OD:	Minimum OD for samples collected
MAX OD:	Maximum OD for samples collected
NO. SPLES. +>10:	Number of samples with OD% greater than 10
NO. SPLES. +>40:	Number of samples with OD% greater than 40
% POS +>10:	Percent of samples with OD% greater than 10
%POS +>40:	Percent of samples with OD% greater than 40
***Salmonella* Level, OD% >10:**	*Salmonella* level using OD% cut-off of 10
***Salmonella* Level, OD%>40:**	*Salmonella* level using OD% cut-off of 40
Lbs. per sq. Ft. Per yr.:	Pounds of liveweight pork generated per square foot of finisher building per year
FEED EFFICIENCY:	Pounds of feed per pound of pork produced

DATE FILL BEGAN:	Date pigs placed into building
DATE FILL ENDED:	Last day that pigs were placed in the building
SEASON OF FILL:	Quarter of the year in which pigs were placed
NO. DAYS TO FILL:	The number of days between "Date fill began" and "Date fill ended"
NO. WEEKS TO FILL:	The "No. days to fill" divided by 7
AUDITA:	"Mgtmt score" added to "No. weeks to fill"
AUDITS:	"No. weeks to fill" subtracted from "Mgtmt score"
MGTMT SCORE:	Add all values to the right of this column except "No. Nursery Sources". If "No. Nursery Sources" >1, then add 1, if 1, then add 0
NO. NURSERY SOURCES:	Number of nurseries used to fill building
PIG FLOW:	Refer to materials and methods
FARM TYPE:	Refer to materials and methods
FEED SYSTEM:	Refer to materials and methods
BAIT:	Refer to materials and methods
VENT. SYSTEM:	Refer to materials and methods
MANURE REMOVAL:	Refer to materials and methods
FLOOR TYPE:	Refer to materials and methods
SHVIS:	"0" if visitors shower in, "1" if not
SHEMP:	"0" if employees shower in, "1" if not
CLEAN BETWEEN GROUPS:	"0" if facility cleaned between groups, "1" if not

DISINFECT BETWEEN GROUPS: "0" if facility disinfected between groups, "1" if not

PQAIII: "0" if PQA Level III Certified, "1" if not

BIRD-PROOFED: "0" if building has bird mesh, "1" if not

DEADS REMOVED DAILY: "0" if dead pigs removed daily from building, "1" if not

DEADS TAKEN OFF-SITE: "0" if dead pigs taken to an off-site location for pickup from farm, "1" if not

BOOT CHANGE BETWEEN BUILDINGS: "0" if change of boots required before entering building, "1" if not

HOSE IN FINISHING BUILDING: "0" if hose in finishing building for use on boots, "1" if not

NOZZLE ON HOSE IN FINISIHING BUILDING: "0" if nozzle on end of hose, "1" if not

BOOT BATH IN FINISHING BUILDING: "0" if boot bath present in building, "1" if not

TRAILER CLEANED AFTER USE: "0" if trailers that transport pigs are cleaned after each use, "1" if not

SEPARATE TRAILER FOR SALES: "0" if separate trailer used for transport of pigs to slaughter, "1" if not

TRANSPORT PERSONNEL IN BUILDING: "0" if personnel who drive trucks to slaughter facilities are allowed into the building, "1"

HOLDBACKS IN FINISHING: "0" if all pigs removed from building or site before next group of pigs brought into building or site, "1" if not

FEED SOURCE: "0" if feed manufactured by toll arrangement, "1" if feed manufactured by farm employees

FEED TYPE: "0" if pelleted feeds used, "1" if not

FARM CODE	DATE SPLED	MONTH SPLED	QTR. SPLED	NO. SPLES.	AVG. OD.	MIN. OD.	MAX. OD.	NO. SPLES. +>10	NO. SPLES. +>40	% POS +>10	% POS +>40	Salmonella Level, OD% >10
A	Dec-94	12	4	36	9.39	0	97	10	3	28%	8%	3
A	Jan-95	1	1	30	4.40	0	75	2	1	7%	3%	1
A	Feb-95	2	1	30	4.30	0	17	5	0	17%	0%	3
A	Mar-95	3	1	30	9.20	0	56	7	2	23%	7%	3
A	May-95	5	2	40	3.30	0	28	3	0	8%	0%	1
A	Jun-95	6	2	30	0.30	0	7	0	0	0%	0%	1
A	Jul-95	7	3	34	5.32	0	35	3	0	9%	0%	1
A	Aug-95	8	3	29	5.72	0	16	3	0	10%	0%	2
A	Sep-95	9	3	57	11.88	0	69	21	4	37%	7%	3
A	Oct-95	10	4	30	5.67	0	21	4	0	13%	0%	2
A	Dec-95	12	4	25	0.72	0	5	0	0	0%	0%	1
B	Jan-95	1	1	30	1.33	0	24	2	0	7%	0%	1
B	Feb-95	2	1	30	1.77	0	16	1	0	3%	0%	1
B	Mar-95	3	1	30	4.30	0	42	4	1	13%	3%	2
B	Apr-95	4	2	29	3.14	0	12	3	0	10%	0%	2
B	May-95	5	2	30	3.90	0	29	3	0	10%	0%	1
B	Jun-95	6	2	30	5.40	0	34	6	0	20%	0%	3
B	Jul-95	7	3	30	4.20	0	17	5	0	17%	0%	3
B	Aug-95	8	3	29	7.00	0	20	8	0	28%	0%	3
B	Sep-95	9	3	30	1.80	0	10	0	0	0%	0%	1
B	Oct-95	10	4	29	8.24	0	32	8	0	28%	0%	3
B	Nov-95	11	4	30	2.83	0	6	0	0	0%	0%	1
B	Dec-95	12	4	30	2.40	0	17	2	0	7%	0%	1
C	Dec-94	12	4	58	7.57	0	118	11	3	19%	5%	3
C	Jan-95	1	1	30	0.00	0	0	0	0	0%	0%	1
C	Jan-95	1	1	30	16.87	0	90	12	5	40%	17%	3
C	Jan-95	1	1	30	0.53	0	11	1	0	3%	0%	1
C	Jan-95	1	1	30	0.00	0	0	0	0	0%	0%	1
C	Feb-95	2	1	30	10.30	0	109	6	2	20%	7%	3
C	Feb-95	2	1	30	20.13	0	130	10	5	33%	17%	3
C	Mar-95	3	1	28	5.57	0	52	3	3	11%	11%	2

FARM CODE	DATE SPLED	MONTH SPLED	QTR. SPLED	NO. SPLES.	AVG. OD.	MIN. OD.	MAX. OD.	NO. SPLES. +>10	NO. SPLES. +>40	% POS +>10	% POS +>40	Salmonella Level, OD% >10
C	Mar-95	3	2	29	1.45	0	14	1	0	3%	0%	1
C	Mar-95	3	1	30	2.90	0	23	4	0	13%	0%	2
C	Apr-95	4	2	29	3.00	0	16	2	0	7%	0%	1
C	Apr-95	4	2	30	8.27	0	28	8	0	27%	0%	3
C	Apr-95	4	2	30	2.73	0	13	3	0	10%	0%	1
C	Apr-95	4	2	29	4.38	0	23	2	0	7%	0%	1
C	May-95	5	2	30	39.83	1	124	22	12	73%	40%	3
C	May-95	5	2	30	47.67	4	137	28	15	93%	50%	3
C	May-95	5	2	29	4.07	0	16	1	0	3%	0%	1
C	May-95	5	2	32	3.38	0	17	2	0	6%	0%	1
C	Jun-95	6	2	30	10.50	0	113	6	2	20%	7%	3
C	Jun-95	6	2	30	2.40	0	13	2	0	7%	0%	1
C	Jun-95	6	3	30	4.93	0	41	3	1	10%	3%	1
C	Jun-95	6	0	30	34.77	0	119	20	10	67%	33%	3
C	Aug-95	8	3	30	52.80	6	113	26	19	87%	63%	3
C	Aug-95	8	3	30	33.10	0	127	17	9	57%	30%	3
C	Sep-95	9	3	30	50.90	4	116	26	16	87%	53%	3
C	Sep-95	9	3	30	51.27	12	132	30	17	100%	57%	3
C	Sep-95	9	3	28	36.32	1	86	22	12	79%	43%	3
C	Oct-95	10	4	30	15.87	0	71	13	4	43%	13%	3
C	Oct-95	10	4	30	34.73	2	93	24	10	80%	33%	3
C	Oct-95	10	4	28	46.71	4	105	25	14	89%	50%	3
C	Nov-95	11	4	31	16.55	0	90	10	6	32%	19%	3
C	Nov-95	11	4	30	6.17	0	92	4	1	13%	3%	2
C	Nov-95	11	4	30	29.00	0	118	18	8	60%	27%	3
C	Nov-95	11	4	26	35.58	1	88	20	10	77%	38%	3
C	Dec-95	12	4	30	47.07	0	104	24	16	80%	53%	3
C	Dec-95	12	4	30	35.90	1	88	24	11	80%	37%	3
F	Jan-95	1	1	30	11.53	0	76	8	5	27%	17%	3
F	Jan-95	1	1	30	0.67	0	5	0	0	0%	0%	1
F	Feb-95	2	1	30	4.87	0	25	2	0	7%	0%	1

FARM CODE	DATE SPLED	MONTH SPLED	QTR. SPLED	NO. SPLES.	AVG. OD.	MIN. OD.	MAX. OD.	NO. SPLES. +>10	NO. SPLES. +>40	% POS +>10	% POS +>40	Salmonella Level, OD% >10
F	Feb-95	2	1	29	0.97	0	4	0	0	0%	0%	1
F	Mar-95	3	1	30	17.97	0	119	14	4	47%	13%	3
F	Mar-95	3	1	30	34.53	0	122	21	7	70%	23%	3
F	Apr-95	4	2	29	1.00	0	7	0	0	0%	0%	1
F	Apr-95	4	2	30	1.63	0	7	0	0	0%	0%	1
F	Jun-95	6	2	30	1.53	0	21	1	0	3%	0%	1
F	Jun-95	6	2	30	0.57	0	5	0	0	0%	0%	1
G	Jan-95	1	1	43	0.05	0	2	0	0	0%	0%	1
G	Feb-95	2	1	38	1.95	0	50	1	0	3%	0%	1
G	Apr-95	4	2	28	0.00	0	0	0	0	0%	0%	1
G	May-95	5	2	38	0.24	0	5	0	0	0%	0%	1
G	Sep-95	9	3	38	1.47	0	12	1	0	3%	0%	1
G	Oct-95	10	4	41	0.41	0	7	0	0	0%	0%	1
G	Nov-95	11	4	40	0.70	0	6	0	0	0%	0%	1
G	Dec-95	12	4	30	0.10	0	3	0	0	0%	0%	1
H	Jan-95	1	1	58	16.41	0	63	33	6	57%	10%	3
H	Feb-95	2	1	58	16.48	0	117	26	3	45%	5%	3
H	Mar-95	3	1	57	6.39	0	42	34	3	60%	5%	3
H	Apr-95	4	2	60	6.23	0	46	11	1	18%	2%	3
H	May-95	5	2	59	6.24	0	31	11	0	19%	0%	3
H	Jun-95	6	2	60	9.78	0	55	11	0	18%	0%	3
H	Jul-95	7	3	60	5.67	0	26	8	0	13%	0%	2
H	Aug-95	8	3	59	5.41	0	35	9	0	15%	0%	3
H	Sep-95	9	3	59	7.81	0	26	14	0	24%	0%	3
H	Oct-95	10	4	60	16.15	0	308	28	0	47%	0%	3
H	Nov-95	11	4	59	9.17	0	32	19	0	32%	0%	3
H	Dec-95	12	4	60	7.17	0	54	14	0	23%	0%	3

FARM CODE	DATE SPLED	MONTH SPLED	QTR. SPLED	Salmonella Level, OD% > 40	Lbs. per sq. ft. per yr.	FEED EFFICIENCY	DATE FILL BEGAN	DATE FILL ENDED	SEASON OF FILL	NO. DAYS TO FILL	NO. WEEKS TO FILL
A	Dec-94	12	4	1	.	.	7/1/94	1/2/95	4	185	26.4
A	Jan-95	1	1	1	.	.	9/1/95	9/20/95	3	0	0.1
A	Feb-95	2	1	1	.	.	12/10/94	12/10/94	4	0	0.1
A	Mar-95	3	1	1	.	.	10/22/94	11/15/94	4	24	3.4
A	May-95	5	2	1	.	.	2/1/95	2/1/95	1	0	0.1
A	Jun-95	6	2	1	.	.	2/1/95	2/28/95	1	27	3.9
A	Jul-95	7	3	1	.	.	2/1/95	2/28/95	1	27	3.9
A	Aug-95	8	3	1	.	.	5/6/95	5/6/95	2	0	0.1
A	Sep-95	9	3	1	.	.	6/26/95	6/26/95	2	0	0.1
A	Oct-95	10	4	1	.	.	7/1/95	7/14/95	3	13	1.9
A	Dec-95	12	4	1	.	.	8/1/95	8/22/95	3	21	3.0
B	Jan-95	1	1	1	.	.	9/1/94	9/1/94	3	0	0.1
B	Feb-95	2	1	1	.	.	10/1/94	10/1/94	4	0	0.1
B	Mar-95	3	1	1	.	.	11/1/94	11/1/94	4	0	0.1
B	Apr-95	4	2	1	.	.	12/1/94	12/1/94	4	0	0.1
B	May-95	5	2	1	.	.	1/1/95	1/1/95	1	0	0.1
B	Jun-95	6	2	1	.	.	2/1/95	2/1/95	1	0	0.1
B	Jul-95	7	3	1	.	.	3/1/95	3/1/95	1	0	0.1
B	Aug-95	8	3	1	.	.	4/1/95	4/1/95	2	0	0.1
B	Sep-95	9	3	1	.	.	5/1/95	5/1/95	2	0	0.1
B	Oct-95	10	4	1	.	.	6/1/95	6/1/95	2	0	0.1
B	Nov-95	11	4	1	.	.	7/1/95	7/1/95	3	0	0.1
B	Dec-95	12	4	1	.	.	8/1/95	8/1/95	3	0	0.1
C	Dec-94	12	4	1	67.87	3.06	8/11/95	8/25/95	3	14	2.0
C	Jan-95	1	1	1	63.98	3.11	7/27/95	8/3/95	3	7	1.0
C	Jan-95	1	1	3	70.74	3.01	9/20/95	9/28/95	3	8	1.1
C	Jan-95	1	1	1	68.14	3.08	9/13/94	9/20/94	3	7	1.0
C	Jan-95	1	1	1	73.75	3.10	9/28/95	10/4/95	3	6	0.9
C	Feb-95	2	1	1	71.61	3.16	10/18/94	11/1/94	4	14	2.0
C	Feb-95	2	1	3	67.77	3.26	10/13/95	10/18/95	4	5	0.7
C	Mar-95	3	1	2	67.61	3.17	11/1/94	11/14/94	4	13	1.9

FARM CODE	DATE SPLED	MONTH SPLED	QTR. SPLED	Salmonella Level, OD% > 40	Lbs. per sq. ft. per yr.	FEED EFFICIENCY	DATE FILL BEGAN	DATE FILL ENDED	SEASON OF FILL	NO. DAYS TO FILL	NO. WEEKS TO FILL
C	Mar-95	3	2	1	70.46	3.15	10/28/94	11/2/94	4	5	0.7
C	Mar-95	3	1	1	67.78	3.24	11/16/94	11/28/94	4	12	1.7
C	Apr-95	4	2	1	68.24	3.08	12/14/94	12/23/94	4	9	1.3
C	Apr-95	4	2	1	68.32	3.11	12/27/94	1/2/95	4	6	0.9
C	Apr-95	4	2	1	67.89	3.19	11/30/94	12/5/94	4	5	0.7
C	Apr-95	4	2	1	65.48	3.18	12/5/94	12/15/94	4	10	1.4
C	May-95	5	2	3	66.14	3.02	2/1/95	2/13/95	1	12	1.7
C	May-95	5	2	3	67.24	2.94	1/23/95	1/31/95	1	8	1.1
C	May-95	5	2	1	67.85	3.10	1/4/95	1/12/95	1	8	1.1
C	May-95	5	2	1	64.56	3.04	1/16/95	1/20/95	1	4	0.6
C	Jun-95	6	2	1	65.28	2.97	3/5/95	3/10/95	1	5	0.7
C	Jun-95	6	2	1	66.66	2.83	2/20/95	2/24/95	1	4	0.6
C	Jun-95	6	3	1	68.84	3.02	2/27/95	3/3/95	1	4	0.6
C	Jun-95	6	0	3	60.38	3.06	3/13/95	3/17/95	1	4	0.6
C	Aug-95	8	3	3	60.07	3.12	5/8/95	5/15/95	2	7	1.0
C	Aug-95	8	3	3	60.16	3.14	5/1/95	5/5/95	2	4	0.6
C	Sep-95	9	3	3	62.52	2.97	5/16/95	5/30/95	2	14	2.0
C	Sep-95	9	3	3	59.00	3.14	4/14/95	4/21/95	2	7	1.0
C	Sep-95	9	3	3	61.40	3.05	5/12/95	5/19/95	2	7	1.0
C	Oct-95	10	4	2	65.52	3.10	6/26/95	6/29/95	2	3	0.4
C	Oct-95	10	4	3	66.28	3.07	6/16/95	6/21/95	2	5	0.7
C	Oct-95	10	4	3	63.14	3.00	6/2/95	6/2/95	2	0	0.1
C	Nov-95	11	4	3	68.57	3.00	7/27/95	8/3/95	3	7	1.0
C	Nov-95	11	4	1	69.79	2.94	7/10/95	7/13/95	3	3	0.4
C	Nov-95	11	4	3	58.57	3.07	7/17/95	7/21/95	3	4	0.6
C	Nov-95	11	4	3	66.07	3.17	8/11/95	8/15/95	3	4	0.6
C	Dec-95	12	4	3	62.63	3.19	9/12/95	9/19/95	3	7	1.0
C	Dec-95	12	4	3	62.26	3.02	8/29/95	9/5/95	3	7	1.0
F	Jan-95	1	1	3	.	.	9/15/94	9/15/94	3	0	0.1
F	Jan-95	1	1	1	.	.	9/15/94	9/15/94	3	0	0.1
F	Feb-95	2	1	1	.	.	10/15/94	10/15/94	4	0	0.1

FARM CODE	DATE SPLED	MONTH SPLED	QTR. SPLED	Salmonella Level, OD% > 40	Lbs. per sq. ft. per yr.	FEED EFFICIENCY	DATE FILL BEGAN	DATE FILL ENDED	SEASON OF FILL	NO. DAYS TO FILL	NO. WEEKS TO FILL
F	Feb-95	2	1	1	.	.	10/15/94	10/15/94	4	0	0.1
F	Mar-95	3	1	2	.	.	12/15/94	12/15/94	4	0	0.1
F	Mar-95	3	1	3	.	.	12/15/94	12/15/94	4	0	0.1
F	Apr-95	4	2	1	.	.	12/1/94	12/1/94	4	0	0.1
F	Apr-95	4	2	1	.	.	12/1/94	12/1/94	4	0	0.1
F	Jun-95	6	2	1	.	.	2/15/95	2/15/95	1	0	0.1
F	Jun-95	6	2	1	.	.	2/15/95	2/15/95	1	0	0.1
G	Jan-95	1	1	1	.	.	8/31/94	9/30/94	3	30	4.3
G	Feb-95	2	1	1	.	.	9/30/94	10/31/94	3	31	4.4
G	Apr-95	4	2	1	.	.	11/15/94	12/31/94	4	46	6.6
G	May-95	5	2	1	.	.	12/15/94	1/15/95	4	31	4.4
G	Sep-95	9	3	1	.	.	12/31/94	2/15/95	4	46	6.6
G	Oct-95	10	4	1	.	.	5/31/95	6/30/95	2	30	4.3
G	Nov-95	11	4	1	.	.	6/1/95	7/1/95	2	30	4.3
G	Dec-95	12	4	1	.	.	7/15/95	8/15/95	3	31	4.4
H	Jan-95	1	1	2	65.00	3.50	7/12/94	8/23/94	3	42	6.0
H	Feb-95	2	1	1	55.00	3.30	8/30/94	10/11/94	3	42	6.0
H	Mar-95	3	1	1	75.80	3.80	10/18/94	11/29/94	4	42	6.0
H	Apr-95	4	2	1	68.60	3.40	12/6/94	1/18/95	4	43	6.1
H	May-95	5	2	1	65.00	3.40	1/24/95	1/31/95	1	7	1.0
H	Jun-95	6	2	1	74.70	3.10	2/12/95	2/22/95	1	10	1.4
H	Jul-95	7	3	1	70.30	3.30	3/15/95	4/9/95	1	25	3.6
H	Aug-95	8	3	1	75.10	3.10	4/9/95	5/1/95	2	22	3.1
H	Sep-95	9	3	1	72.90	3.10	5/1/95	5/22/95	2	25	3.6
H	Oct-95	10	4	1	76.40	3.30	5/22/95	6/14/95	2	22	3.1
H	Nov-95	11	4	1	67.80	3.10	6/14/95	7/3/95	2	21	3.0
H	Dec-95	12	4	1	70.40	3.40	7/5/95	7/24/95	3	23	3.3

FARM CODE	DATE SPLED	MONTH SPLED	QTR. SPLED	AUDITA	AUDITS	MGTMT SCOR	NO. NURSERY SOURCES	PIG FLOW	FARM TYPE	FARM DESIGN	FEED SYSTEM
A	Dec-94	12	4	56.4	3.6	30.0	1	4	3	0	0
A	Jan-95	1	1	28.1	27.9	28.0	1	4	3	0	0
A	Feb-95	2	1	29.1	28.9	29.0	1	4	3	0	0
A	Mar-95	3	1	31.4	24.6	28.0	1	4	3	0	0
A	May-95	5	2	22.1	21.9	22.0	1	1	3	0	0
A	Jun-95	6	2	31.9	24.1	29.0	2	4	3	0	0
A	Jul-95	7	3	31.9	24.1	28.0	1	4	3	0	0
A	Aug-95	8	3	24.1	23.9	24.0	1	1	3	0	0
A	Sep-95	9	3	22.1	21.9	22.0	1	1	3	0	0
A	Oct-95	10	4	30.9	27.1	29.0	1	4	3	0	0
A	Dec-95	12	4	32.0	26.0	29.0	1	4	3	0	0
B	Jan-95	1	1	15.1	14.9	15.0	1	2	2	0	0
B	Feb-95	2	1	15.1	14.9	15.0	1	2	2	0	0
B	Mar-95	3	1	14.1	13.9	14.0	1	2	1	0	0
B	Apr-95	4	2	14.1	13.9	14.0	1	2	1	0	0
B	May-95	5	2	14.1	13.9	14.0	1	2	1	0	0
B	Jun-95	6	2	21.1	20.9	21.0	1	4	1	0	0
B	Jul-95	7	3	14.1	13.9	14.0	1	2	1	0	0
B	Aug-95	8	3	16.1	15.9	16.0	1	0	1	0	0
B	Sep-95	9	3	14.1	13.9	14.0	1	2	1	0	0
B	Oct-95	10	4	14.1	13.9	14.0	1	2	1	0	0
B	Nov-95	11	4	14.1	13.9	14.0	1	2	1	0	0
B	Dec-95	12	4	14.1	13.9	14.0	1	2	1	0	0
C	Dec-94	12	4	11.0	7.0	10.0	4	0	2	0	0
C	Jan-95	1	1	15.0	13.0	15.0	4	4	2	0	0
C	Jan-95	1	1	15.1	12.9	15.0	4	4	2	0	0
C	Jan-95	1	1	15.0	13.0	15.0	4	4	2	0	0
C	Jan-95	1	1	9.9	8.1	10.0	4	0	2	0	0
C	Feb-95	2	1	16.0	12.0	15.0	4	4	2	0	0
C	Feb-95	2	1	14.7	13.3	15.0	4	4	2	0	0
C	Mar-95	3	1	10.9	7.1	10.0	4	0	2	0	0

FARM CODE	DATE SPLED	MONTH SPLED	QTR. SPLED	AUDITA	AUDITS	MGTMT SCOR	NO. NURSERY SOURCES	PIG FLOW	FARM TYPE	FARM DESIGN	FEED SYSTEM
C	Mar-95	3	2	9.7	8.3	10.0	4	0	2	0	0
C	Mar-95	3	1	10.7	7.3	10.0	4	0	2	0	0
C	Apr-95	4	2	10.3	7.7	10.0	4	0	2	0	0
C	Apr-95	4	2	14.9	13.1	15.0	4	4	2	0	0
C	Apr-95	4	2	9.7	8.3	10.0	4	0	2	0	0
C	Apr-95	4	2	10.4	7.6	10.0	4	0	2	0	0
C	May-95	5	2	15.7	12.3	15.0	4	4	2	0	0
C	May-95	5	2	15.1	12.9	15.0	4	4	2	0	0
C	May-95	5	2	10.1	7.9	10.0	4	0	2	0	0
C	May-95	5	2	14.6	13.4	15.0	4	4	2	0	0
C	Jun-95	6	2	14.7	13.3	15.0	4	4	2	0	0
C	Jun-95	6	2	14.6	13.4	15.0	4	4	2	0	0
C	Jun-95	6	3	9.6	8.4	10.0	4	0	2	0	0
C	Jun-95	6	0	14.6	13.4	15.0	4	4	2	0	0
C	Aug-95	8	3	10.0	8.0	10.0	4	0	2	0	0
C	Aug-95	8	3	9.6	8.4	10.0	4	0	2	0	0
C	Sep-95	9	3	11.0	7.0	10.0	4	0	2	0	0
C	Sep-95	9	3	10.0	8.0	10.0	4	0	2	0	0
C	Sep-95	9	3	10.0	8.0	10.0	4	0	2	0	0
C	Oct-95	10	4	14.4	13.6	15.0	4	4	2	0	0
C	Oct-95	10	4	14.7	13.3	15.0	4	4	2	0	0
C	Oct-95	10	4	9.1	8.9	10.0	4	0	2	0	0
C	Nov-95	11	4	15.0	13.0	15.0	4	4	2	0	0
C	Nov-95	11	4	9.4	8.6	10.0	4	0	2	0	0
C	Nov-95	11	4	14.6	13.4	15.0	4	4	2	0	0
C	Nov-95	11	4	9.6	8.4	10.0	4	0	2	0	0
C	Dec-95	12	4	10.0	8.0	10.0	4	0	2	0	0
C	Dec-95	12	4	15.0	13.0	15.0	4	4	2	0	0
F	Jan-95	1	1	5.1	4.9	5.0	1	1	0	0	2
F	Jan-95	1	1	5.1	4.9	5.0	1	1	0	0	2
F	Feb-95	2	1	5.1	4.9	5.0	1	1	0	0	2

FARM CODE	DATE SPLED	MONTH SPLED	QTR. SPLED	AUDITA	AUDITS	MGTMT SCOR	NO. NURSERY SOURCES	PIG FLOW	FARM TYPE	FARM DESIGN	FEED SYSTEM
F	Feb-95	2	1	5.1	4.9	5.0	1	1	0	0	2
F	Mar-95	3	1	5.1	4.9	5.0	1	1	0	0	2
F	Mar-95	3	1	5.1	4.9	5.0	1	1	0	0	2
F	Apr-95	4	2	5.1	4.9	5.0	1	1	0	0	2
F	Apr-95	4	2	5.1	4.9	5.0	1	1	0	0	2
F	Jun-95	6	2	5.1	4.9	5.0	1	1	0	0	2
F	Jun-95	6	2	5.1	4.9	5.0	1	1	0	0	2
G	Jan-95	1	1	13.3	4.7	9.0	1	4	1	0	1
G	Feb-95	2	1	13.4	4.6	9.0	1	4	1	0	1
G	Apr-95	4	2	15.6	2.4	9.0	1	4	1	0	1
G	May-95	5	2	13.4	4.6	9.0	1	4	1	0	1
G	Sep-95	9	3	15.6	2.4	9.0	1	4	1	0	1
G	Oct-95	10	4	13.3	4.7	9.0	1	4	1	0	1
G	Nov-95	11	4	13.3	4.7	9.0	1	4	1	0	1
G	Dec-95	12	4	13.4	4.6	9.0	1	4	1	0	1
H	Jan-95	1	1	15.0	3.0	9.0	1	1	1	0	2
H	Feb-95	2	1	15.0	3.0	9.0	1	1	1	0	2
H	Mar-95	3	1	15.0	3.0	9.0	1	1	1	0	2
H	Apr-95	4	2	15.1	2.9	9.0	1	1	1	0	2
H	May-95	5	2	10.0	8.0	9.0	1	1	1	0	2
H	Jun-95	6	2	10.4	7.6	9.0	1	1	1	0	2
H	Jul-95	7	3	12.6	5.4	9.0	1	1	1	0	2
H	Aug-95	8	3	12.1	5.9	9.0	1	1	1	0	2
H	Sep-95	9	3	12.6	5.4	9.0	1	1	1	0	2
H	Oct-95	10	4	12.1	5.9	9.0	1	1	1	0	2
H	Nov-95	11	4	12.0	6.0	9.0	1	1	1	0	2
H	Dec-95	12	4	12.3	5.7	9.0	1	1	1	0	2

FARM CODE	DATE SPLED	MONTH SPLED	QTR. SPLED	BAIT	VENT. SYSTEM	MANURE REMOVAL	FLOOR TYPE	SHVIS	SHEMP	PERFEN	CLEAN BETWEEN GROUPS
A	Dec-94	12	4	1	1	2	3	1	1	1	1
A	Jan-95	1	1	1	0	2	3	1	1	1	0
A	Feb-95	2	1	1	0	2	3	1	1	1	0
A	Mar-95	3	1	1	0	2	3	1	1	1	0
A	May-95	5	2	1	0	0	1	1	1	1	1
A	Jun-95	6	2	1	0	2	3	1	1	1	0
A	Jul-95	7	3	1	0	2	3	1	1	1	0
A	Aug-95	8	3	1	0	2	3	1	1	1	0
A	Sep-95	9	3	1	0	0	1	1	1	1	1
A	Oct-95	10	4	1	0	2	3	1	1	1	1
A	Dec-95	12	4	1	0	2	3	1	1	1	1
B	Jan-95	1	1	1	0	1	0	1	1	1	0
B	Feb-95	2	1	1	0	1	0	1	1	1	0
B	Mar-95	3	1	1	0	1	0	1	1	1	0
B	Apr-95	4	2	1	0	1	0	1	1	1	0
B	May-95	5	2	1	0	1	0	1	1	1	0
B	Jun-95	6	2	1	0	2	3	1	1	1	0
B	Jul-95	7	3	1	0	1	0	1	1	1	0
B	Aug-95	8	3	1	0	2	3	1	1	1	0
B	Sep-95	9	3	1	0	1	0	1	1	1	0
B	Oct-95	10	4	1	0	1	0	1	1	1	0
B	Nov-95	11	4	1	0	1	0	1	1	1	0
B	Dec-95	12	4	1	0	1	0	1	1	1	0
C	Dec-94	12	4	0	0	3	0	0	0	1	0
C	Jan-95	1	1	0	0	3	0	0	0	1	0
C	Jan-95	1	1	0	0	3	0	0	0	1	0
C	Jan-95	1	1	0	0	3	0	0	0	1	0
C	Jan-95	1	1	0	0	3	0	0	0	1	0
C	Feb-95	2	1	0	0	3	0	0	0	1	0
C	Feb-95	2	1	0	0	3	0	0	0	1	0
C	Mar-95	3	1	0	0	3	0	0	0	1	0

FARM CODE	DATE SPLED	MONTH SPLED	QTR. SPLED	BAIT	VENT. SYSTEM	MANURE REMOVAL	FLOOR TYPE	SHVIS	SHEMP	PERFEN	CLEAN BETWEEN GROUPS
C	Mar-95	3	2	0	0	3	0	0	0	1	0
C	Mar-95	3	1	0	0	3	0	0	0	1	0
C	Apr-95	4	2	0	0	3	0	0	0	1	0
C	Apr-95	4	2	0	0	3	0	0	0	1	0
C	Apr-95	4	2	0	0	3	0	0	0	1	0
C	Apr-95	4	2	0	0	3	0	0	0	1	0
C	May-95	5	2	0	0	3	0	0	0	1	0
C	May-95	5	2	0	0	3	0	0	0	1	0
C	May-95	5	2	0	0	3	0	0	0	1	0
C	May-95	5	2	0	0	3	0	0	0	1	0
C	Jun-95	6	2	0	0	3	0	0	0	1	0
C	Jun-95	6	2	0	0	3	0	0	0	1	0
C	Jun-95	6	3	0	0	3	0	0	0	1	0
C	Jun-95	6	0	0	0	3	0	0	0	1	0
C	Aug-95	8	3	0	0	3	0	0	0	1	0
C	Aug-95	8	3	0	0	3	0	0	0	1	0
C	Sep-95	9	3	0	0	3	0	0	0	1	0
C	Sep-95	9	3	0	0	3	0	0	0	1	0
C	Sep-95	9	3	0	0	3	0	0	0	1	0
C	Oct-95	10	4	0	0	3	0	0	0	1	0
C	Oct-95	10	4	0	0	3	0	0	0	1	0
C	Oct-95	10	4	0	0	3	0	0	0	1	0
C	Nov-95	11	4	0	0	3	0	0	0	1	0
C	Nov-95	11	4	0	0	3	0	0	0	1	0
C	Nov-95	11	4	0	0	3	0	0	0	1	0
C	Nov-95	11	4	0	0	3	0	0	0	1	0
C	Dec-95	12	4	0	0	3	0	0	0	1	0
C	Dec-95	12	4	0	0	3	0	0	0	1	0
F	Jan-95	1	1	0	0	0	0	0	0	1	0
F	Jan-95	1	1	0	0	0	0	0	0	1	0
F	Feb-95	2	1	0	0	0	0	0	0	1	0

FARM CODE	DATE SPLED	MONTH SPLED	QTR. SPLED	BAIT	VENT. SYSTEM	MANURE REMOVAL	FLOOR TYPE	SHVIS	SHEMP	PERFEN	CLEAN BETWEEN GROUPS
F	Feb-95	2	1	0	0	0	0	0	0	1	0
F	Mar-95	3	1	0	0	0	0	0	0	1	0
F	Mar-95	3	1	0	0	0	0	0	0	1	0
F	Apr-95	4	2	0	0	0	0	0	0	1	0
F	Apr-95	4	2	0	0	0	0	0	0	1	0
F	Jun-95	6	2	0	0	0	0	0	0	1	0
F	Jun-95	6	2	0	0	0	0	0	0	1	0
G	Jan-95	1	1	0	0	1	0	0	0	1	0
G	Feb-95	2	1	0	0	1	0	0	0	1	0
G	Apr-95	4	2	0	0	1	0	0	0	1	0
G	May-95	5	2	0	0	1	0	0	0	1	0
G	Sep-95	9	3	0	0	1	0	0	0	1	0
G	Oct-95	10	4	0	0	1	0	0	0	1	0
G	Nov-95	11	4	0	0	1	0	0	0	1	0
G	Dec-95	12	4	0	0	1	0	0	0	1	0
H	Jan-95	1	1	0	0	0	0	0	0	1	0
H	Feb-95	2	1	0	0	0	0	0	0	1	0
H	Mar-95	3	1	0	0	0	0	0	0	1	0
H	Apr-95	4	2	0	0	0	0	0	0	1	0
H	May-95	5	2	0	0	0	0	0	0	1	0
H	Jun-95	6	2	0	0	0	0	0	0	1	0
H	Jul-95	7	3	0	0	0	0	0	0	1	0
H	Aug-95	8	3	0	0	0	0	0	0	1	0
H	Sep-95	9	3	0	0	0	0	0	0	1	0
H	Oct-95	10	4	0	0	0	0	0	0	1	0
H	Nov-95	11	4	0	0	0	0	0	0	1	0
H	Dec-95	12	4	0	0	0	0	0	0	1	0

FARM CODE	DATE SPLED	MONTH SPLED	QTR. SPLED	DISINFECT BETWEEN GROUPS	PQAIII	BIRD-PROOFED	DEADS REMOVED DAILY	DEADS TAKEN OFF-SITE	BOOT CHANGE BETWEEN BUILDINGS	HOSE IN FINISHING BUILDING
A	Dec-94	12	4	1	0	1	0	0	1	1
A	Jan-95	1	1	1	0	1	0	0	1	1
A	Feb-95	2	1	1	0	1	1	0	1	1
A	Mar-95	3	1	1	0	1	0	0	1	1
A	May-95	5	2	1	0	1	0	0	1	1
A	Jun-95	6	2	1	0	1	0	0	1	1
A	Jul-95	7	3	1	0	1	0	0	1	1
A	Aug-95	8	3	1	0	1	0	0	1	1
A	Sep-95	9	3	1	0	1	0	0	1	1
A	Oct-95	10	4	1	0	1	0	0	1	1
A	Dec-95	12	4	1	0	1	0	0	1	1
B	Jan-95	1	1	0	0	0	0	0	0	0
B	Feb-95	2	1	0	0	0	0	0	0	0
B	Mar-95	3	1	0	0	0	0	0	0	0
B	Apr-95	4	2	0	0	0	0	0	0	0
B	May-95	5	2	0	0	0	0	0	0	0
B	Jun-95	6	2	0	0	0	0	0	0	0
B	Jul-95	7	3	0	0	0	0	0	0	0
B	Aug-95	8	3	0	0	0	0	0	0	0
B	Sep-95	9	3	0	0	0	0	0	0	0
B	Oct-95	10	4	0	0	0	0	0	0	0
B	Nov-95	11	4	0	0	0	0	0	0	0
B	Dec-95	12	4	0	0	0	0	0	0	0
C	Dec-94	12	4	0	0	0	1	0	1	0
C	Jan-95	1	1	0	0	0	1	0	1	0
C	Jan-95	1	1	0	0	0	1	0	1	0
C	Jan-95	1	1	0	0	0	1	0	1	0
C	Jan-95	1	1	0	0	0	1	0	1	0
C	Feb-95	2	1	0	0	0	1	0	1	0
C	Feb-95	2	1	0	0	0	1	0	1	0
C	Mar-95	3	1	0	0	0	1	0	1	0

FARM CODE	DATE SPLED	MONTH SPLED	QTR. SPLED	DISINFECT BETWEEN GROUPS	PQAIII	BIRD-PROOFED	DEADS REMOVED DAILY	DEADS TAKEN OFF-SITE	BOOT CHANGE BETWEEN BUILDINGS	HOSE IN FINISHING BUILDING
C	Mar-95	3	2	0	0	0	1	0	1	0
C	Mar-95	3	1	0	0	0	1	0	1	0
C	Apr-95	4	2	0	0	0	1	0	1	0
C	Apr-95	4	2	0	0	0	1	0	1	0
C	Apr-95	4	2	0	0	0	1	0	1	0
C	Apr-95	4	2	0	0	0	1	0	1	0
C	May-95	5	2	0	0	0	1	0	1	0
C	May-95	5	2	0	0	0	1	0	1	0
C	May-95	5	2	0	0	0	1	0	1	0
C	May-95	5	2	0	0	0	1	0	1	0
C	Jun-95	6	2	0	0	0	1	0	1	0
C	Jun-95	6	2	0	0	0	1	0	1	0
C	Jun-95	6	3	0	0	0	1	0	1	0
C	Jun-95	6	0	0	0	0	1	0	1	0
C	Aug-95	8	3	0	0	0	1	0	1	0
C	Aug-95	8	3	0	0	0	1	0	1	0
C	Sep-95	9	3	0	0	0	1	0	1	0
C	Sep-95	9	3	0	0	0	1	0	1	0
C	Sep-95	9	3	0	0	0	1	0	1	0
C	Oct-95	10	4	0	0	0	1	0	1	0
C	Oct-95	10	4	0	0	0	1	0	1	0
C	Oct-95	10	4	0	0	0	1	0	1	0
C	Nov-95	11	4	0	0	0	1	0	1	0
C	Nov-95	11	4	0	0	0	1	0	1	0
C	Nov-95	11	4	0	0	0	1	0	1	0
C	Nov-95	11	4	0	0	0	1	0	1	0
C	Dec-95	12	4	0	0	0	1	0	1	0
C	Dec-95	12	4	0	0	0	1	0	1	0
F	Jan-95	1	1	0	0	0	0	0	0	0
F	Jan-95	1	1	0	0	0	0	0	0	0
F	Feb-95	2	1	0	0	0	0	0	0	0

FARM CODE	DATE SPLED	MONTH SPLED	QTR. SPLED	DISINFECT BETWEEN GROUPS	PQAIII	BIRD-PROOFED	DEADS REMOVED DAILY	DEADS TAKEN OFF-SITE	BOOT CHANGE BETWEEN BUILDINGS	HOSE IN FINISHING BUILDING
F	Feb-95	2	1	0	0	0	0	0	0	0
F	Mar-95	3	1	0	0	0	0	0	0	0
F	Mar-95	3	1	0	0	0	0	0	0	0
F	Apr-95	4	2	0	0	0	0	0	0	0
F	Apr-95	4	2	0	0	0	0	0	0	0
F	Jun-95	6	2	0	0	0	0	0	0	0
F	Jun-95	6	2	0	0	0	0	0	0	0
G	Jan-95	1	1	0	0	0	0	0	0	0
G	Feb-95	2	1	0	0	0	0	0	0	0
G	Apr-95	4	2	0	0	0	0	0	0	0
G	May-95	5	2	0	0	0	0	0	0	0
G	Sep-95	9	3	0	0	0	0	0	0	0
G	Oct-95	10	4	0	0	0	0	0	0	0
G	Nov-95	11	4	0	0	0	0	0	0	0
G	Dec-95	12	4	0	0	0	0	0	0	0
H	Jan-95	1	1	0	0	0	0	0	1	0
H	Feb-95	2	1	0	0	0	0	0	1	0
H	Mar-95	3	1	0	0	0	0	0	1	0
H	Apr-95	4	2	0	0	0	0	0	1	0
H	May-95	5	2	0	0	0	0	0	1	0
H	Jun-95	6	2	0	0	0	0	0	1	0
H	Jul-95	7	3	0	0	0	0	0	1	0
H	Aug-95	8	3	0	0	0	0	0	1	0
H	Sep-95	9	3	0	0	0	0	0	1	0
H	Oct-95	10	4	0	0	0	0	0	1	0
H	Nov-95	11	4	0	0	0	0	0	1	0
H	Dec-95	12	4	0	0	0	0	0	1	0

FARM CODE	DATE SPLED	MONTH SPLED	QTR. SPLED	NOZZLE ON HOSE IN FINISHING BUILDING	BOOT BATH IN FINISHING BUILDING	TRAILER CLEANED BEFORE USE	TRAILER CLEANED AFTER USE	SEPARATE TRAILER FOR SALES	TRANSPORT PERSONNEL IN BUILDING
A	Dec-94	12	4	1	1	1	1	1	1
A	Jan-95	1	1	1	1	1	1	1	1
A	Feb-95	2	1	1	1	1	1	1	1
A	Mar-95	3	1	1	1	1	1	1	1
A	May-95	5	2	1	1	1	1	1	1
A	Jun-95	6	2	1	1	1	1	1	1
A	Jul-95	7	3	1	1	1	1	1	1
A	Aug-95	8	3	1	1	1	1	1	1
A	Sep-95	9	3	1	1	1	1	1	1
A	Oct-95	10	4	1	1	1	1	1	1
A	Dec-95	12	4	1	1	1	1	1	1
B	Jan-95	1	1	0	0	1	1	1	1
B	Feb-95	2	1	0	0	1	1	1	1
B	Mar-95	3	1	0	0	1	1	1	1
B	Apr-95	4	2	0	0	1	1	1	1
B	May-95	5	2	0	0	1	1	1	1
B	Jun-95	6	2	0	0	1	1	1	1
B	Jul-95	7	3	0	0	1	1	1	1
B	Aug-95	8	3	0	0	1	1	1	1
B	Sep-95	9	3	0	0	1	1	1	1
B	Oct-95	10	4	0	0	1	1	1	1
B	Nov-95	11	4	0	0	1	1	1	1
B	Dec-95	12	4	0	0	1	1	1	1
C	Dec-94	12	4	1	0	0	0	0	0
C	Jan-95	1	1	1	0	0	0	0	0
C	Jan-95	1	1	1	0	0	0	0	0
C	Jan-95	1	1	1	0	0	0	0	0
C	Jan-95	1	1	1	0	0	0	0	0
C	Feb-95	2	1	1	0	0	0	0	0
C	Feb-95	2	1	1	0	0	0	0	0
C	Mar-95	3	1	1	0	0	0	0	0

FARM CODE	DATE SPLED	MONTH SPLED	QTR. SPLED	NOZZLE ON HOSE IN FINISHING BUILDING	BOOT BATH IN FINISHING BUILDING	TRAILER CLEANED BEFORE USE	TRAILER CLEANED AFTER USE	SEPARATE TRAILER FOR SALES	TRANSPORT PERSONNEL IN BUILDING
C	Mar-95	3	2	1	0	0	0	0	0
C	Mar-95	3	1	1	0	0	0	0	0
C	Apr-95	4	2	1	0	0	0	0	0
C	Apr-95	4	2	1	0	0	0	0	0
C	Apr-95	4	2	1	0	0	0	0	0
C	Apr-95	4	2	1	0	0	0	0	0
C	May-95	5	2	1	0	0	0	0	0
C	May-95	5	2	1	0	0	0	0	0
C	May-95	5	2	1	0	0	0	0	0
C	May-95	5	2	1	0	0	0	0	0
C	Jun-95	6	2	1	0	0	0	0	0
C	Jun-95	6	2	1	0	0	0	0	0
C	Jun-95	6	3	1	0	0	0	0	0
C	Jun-95	6	0	1	0	0	0	0	0
C	Aug-95	8	3	1	0	0	0	0	0
C	Aug-95	8	3	1	0	0	0	0	0
C	Sep-95	9	3	1	0	0	0	0	0
C	Sep-95	9	3	1	0	0	0	0	0
C	Sep-95	9	3	1	0	0	0	0	0
C	Oct-95	10	4	1	0	0	0	0	0
C	Oct-95	10	4	1	0	0	0	0	0
C	Oct-95	10	4	1	0	0	0	0	0
C	Nov-95	11	4	1	0	0	0	0	0
C	Nov-95	11	4	1	0	0	0	0	0
C	Nov-95	11	4	1	0	0	0	0	0
C	Nov-95	11	4	1	0	0	0	0	0
C	Dec-95	12	4	1	0	0	0	0	0
C	Dec-95	12	4	1	0	0	0	0	0
F	Jan-95	1	1	0	0	0	0	0	0
F	Jan-95	1	1	0	0	0	0	0	0
F	Feb-95	2	1	0	0	0	0	0	0

FARM CODE	DATE SPLED	MONTH SPLED	QTR. SPLED	NOZZLE ON HOSE IN FINISHING BUILDING	BOOT BATH IN FINISHING BUILDING	TRAILER CLEANED BEFORE USE	TRAILER CLEANED AFTER USE	SEPARATE TRAILER FOR SALES	TRANSPORT PERSONNEL IN BUILDING
F	Feb-95	2	1	0	0	0	0	0	0
F	Mar-95	3	1	0	0	0	0	0	0
F	Mar-95	3	1	0	0	0	0	0	0
F	Apr-95	4	2	0	0	0	0	0	0
F	Apr-95	4	2	0	0	0	0	0	0
F	Jun-95	6	2	0	0	0	0	0	0
F	Jun-95	6	2	0	0	0	0	0	0
G	Jan-95	1	1	0	0	0	0	0	0
G	Feb-95	2	1	0	0	0	0	0	0
G	Apr-95	4	2	0	0	0	0	0	0
G	May-95	5	2	0	0	0	0	0	0
G	Sep-95	9	3	0	0	0	0	0	0
G	Oct-95	10	4	0	0	0	0	0	0
G	Nov-95	11	4	0	0	0	0	0	0
G	Dec-95	12	4	0	0	0	0	0	0
H	Jan-95	1	1	0	0	0	0	0	1
H	Feb-95	2	1	0	0	0	0	0	1
H	Mar-95	3	1	0	0	0	0	0	1
H	Apr-95	4	2	0	0	0	0	0	1
H	May-95	5	2	0	0	0	0	0	1
H	Jun-95	6	2	0	0	0	0	0	1
H	Jul-95	7	3	0	0	0	0	0	1
H	Aug-95	8	3	0	0	0	0	0	1
H	Sep-95	9	3	0	0	0	0	0	1
H	Oct-95	10	4	0	0	0	0	0	1
H	Nov-95	11	4	0	0	0	0	0	1
H	Dec-95	12	4	0	0	0	0	0	1

FARM CODE	DATE SPLED	MONTH SPLED	QTR. SPLED	HOLDBACKS IN FINISHING	FEED SOURCE	FEED TYPE	LAST
A	Dec-94	12	4	1	0	1	1
A	Jan-95	1	1	1	0	1	1
A	Feb-95	2	1	1	0	1	1
A	Mar-95	3	1	1	0	1	1
A	May-95	5	2	1	0	1	1
A	Jun-95	6	2	1	0	1	1
A	Jul-95	7	3	1	0	1	1
A	Aug-95	8	3	0	0	1	1
A	Sep-95	9	3	1	0	1	1
A	Oct-95	10	4	1	0	1	1
A	Dec-95	12	4	1	0	1	1
B	Jan-95	1	1	0	1	1	1
B	Feb-95	2	1	0	1	1	1
B	Mar-95	3	1	0	1	1	1
B	Apr-95	4	2	0	1	1	1
B	May-95	5	2	0	1	1	1
B	Jun-95	6	2	1	1	1	1
B	Jul-95	7	3	0	1	1	1
B	Aug-95	8	3	0	1	1	1
B	Sep-95	9	3	0	1	1	1
B	Oct-95	10	4	0	1	1	1
B	Nov-95	11	4	0	1	1	1
B	Dec-95	12	4	0	1	1	1
C	Dec-94	12	4	0	0	0	1
C	Jan-95	1	1	1	0	0	1
C	Jan-95	1	1	1	0	0	1
C	Jan-95	1	1	1	0	0	1
C	Jan-95	1	1	0	0	0	1
C	Feb-95	2	1	1	0	0	1
C	Feb-95	2	1	1	0	0	1
C	Mar-95	3	1	0	0	0	1

FARM CODE	DATE SPLED	MONTH SPLED	QTR. SPLED	HOLDBACKS IN FINISHING	FEED SOURCE	FEED TYPE	LAST
C	Mar-95	3	2	0	0	0	1
C	Mar-95	3	1	0	0	0	1
C	Apr-95	4	2	0	0	0	1
C	Apr-95	4	2	1	0	0	1
C	Apr-95	4	2	0	0	0	1
C	Apr-95	4	2	0	0	0	1
C	May-95	5	2	1	0	0	1
C	May-95	5	2	1	0	0	1
C	May-95	5	2	0	0	0	1
C	May-95	5	2	1	0	0	1
C	Jun-95	6	2	1	0	0	1
C	Jun-95	6	2	1	0	0	1
C	Jun-95	6	3	0	0	0	1
C	Jun-95	6	0	1	0	0	1
C	Aug-95	8	3	0	0	0	1
C	Aug-95	8	3	0	0	0	1
C	Sep-95	9	3	0	0	0	1
C	Sep-95	9	3	0	0	0	1
C	Sep-95	9	3	0	0	0	1
C	Oct-95	10	4	1	0	0	1
C	Oct-95	10	4	1	0	0	1
C	Oct-95	10	4	0	0	0	1
C	Nov-95	11	4	1	0	0	1
C	Nov-95	11	4	0	0	0	1
C	Nov-95	11	4	1	0	0	1
C	Nov-95	11	4	0	0	0	1
C	Dec-95	12	4	0	0	0	1
C	Dec-95	12	4	1	0	0	1
F	Jan-95	1	1	0	0	1	1
F	Jan-95	1	1	0	0	1	1
F	Feb-95	2	1	0	0	1	1

FARM CODE	DATE SPLED	MONTH SPLED	QTR. SPLED	HOLDBACKS IN FINISHING	FEED SOURCE	FEED TYPE	LAST
F	Feb-95	2	1	0	0	1	1
F	Mar-95	3	1	0	0	1	1
F	Mar-95	3	1	0	0	1	1
F	Apr-95	4	2	0	0	1	1
F	Apr-95	4	2	0	0	1	1
F	Jun-95	6	2	0	0	1	1
F	Jun-95	6	2	0	0	1	1
G	Jan-95	1	1	0	0	1	1
G	Feb-95	2	1	0	0	1	1
G	Apr-95	4	2	0	0	1	1
G	May-95	5	2	0	0	1	1
G	Sep-95	9	3	0	0	1	1
G	Oct-95	10	4	0	0	1	1
G	Nov-95	11	4	0	0	1	1
G	Dec-95	12	4	0	0	1	1
H	Jan-95	1	1	1	0	1	1
H	Feb-95	2	1	1	0	1	1
H	Mar-95	3	1	1	0	1	1
H	Apr-95	4	2	1	0	1	1
H	May-95	5	2	1	0	1	1
H	Jun-95	6	2	1	0	1	1
H	Jul-95	7	3	1	0	1	1
H	Aug-95	8	3	1	0	1	1
H	Sep-95	9	3	1	0	1	1
H	Oct-95	10	4	1	0	1	1
H	Nov-95	11	4	1	0	1	1
H	Dec-95	12	4	1	0	1	1

REFERENCES CITED

Alexander, T. J. L. and D. L. Harris (1992). Methods of Disease Control. Diseases of Swine. I. S. University. Ames, IA, Iowa State University Press: 808-836.

Allred, J. N., J. W. Walker, V. C. Beal and F. W. Germaine (1967). "A survey to determine the *Salmonella* contamination rate in livestock and poultry feeds." JAVMA **151**(12): 1857-1860.

Bager, F. and D. L. Baggesen (1992). Epidemiological typing of porcine *Salmonella* isolates. Ploufragan/Saint-brieuc - France.

Bager, F. and J. Petersen (1991). "Sensitivity and specificity of different methods for the isolation of *Salmonella* from pigs." Acta Vet. Scand. **32**: 437-481.

Baggesen, D. L., J. Dahl, A. Wingstrand and B. Nielsen (1996). Critical control points (CCP) in pig herds in relation to subclinical *Salmonella* infections. 14th Congress Int Pig Vet Soc, Bologna, Italy.

Bailey, T. M. and P. M. Schantz (1986). "Trichinosis surveillance, United States, 1986." MMWR. CDC Surveilance Summaries **37**(ss5): 1-8.

Baum, D. H., B. Nielsen, P. J. Fedorka-Cray, D. L. Harris and K. Ferris (1996). Diagnosis of *Salmonella* by ELISA. 100 Ann Mtg United States Animal Health Association, Little Rock, AR.

Bean, N. H. and M. E. Potter (1992). *Salmonella* serotypes from human serotypes, January 1991 through December 1991. 96th Annual Meeting United States Animal Health Association, Louisville, Kentucky.

Bean, N. H. and M. E. Potter (1994). "*Salmonella* serotypes from human sources January 1992 through December 1992." Proc. U. S. Animal Health Assoc. **97**: 439-442.

Berends, B. R., H. A. P. Urlings, J. M. A. Snijders and F. Van Knapen (1996). "Identification and quantification of risk factors in animal management and transport regarding *Salmonella* spp. in pigs." Intl. J. Food Microbiol. **30**:37-53.

Billy, T. J. (1997). Impact of plant HACCP systems on food animal production - The FSIS perspective. Ann Mtg Livest Conser Inst, Columbus, OH.

Brumm, M. C. (1993). Pig flow - managing inventory for optimum density. 24th Annual Meeting AASP, Kansas City, MO.

CDC. (1991). "*Trichinella spiralis* infection - United States, 1990." Morbid Mortal Wkly Rpt **40**(4): 57-60.

Childers, A. B., E. E. Keahey and A. W. Kotula (1977). "Reduction of *Salmonella* and fecal contamination of pork during swine slaughter." J Am Vet Med Assoc **171**(11): 1161-1164.

Corlett, D. A. and M. D. Pierson (1992). HACCP: Definitions and Principles. HACCP. Principles and Applications. M. D. Pierson and J. D. A. Corlett. New York, NY, Chapman and Hall: 6,7,169-176.

Corwin, R. M. and T. B. Stewart (1992). Internal Parasites. Diseases of Swine. A. D. Leman, B. E. Straw, W. L. Mengling, S. D'Allaire and D. J. Taylor. Ames, IA, Iowa State University Press: 718-734.

Crowe, C. K., D. L. Harris, L. P. Elliot, E. R. Wilson and B. S. Wiseman (1996). "A possible relationship between low facility dust and endotoxin levels and improved growth rates in pigs reared by Isowean™." Swine Health and Production **4**(5): 231-236.

Currier, M., M. Singleton, J. Lee and D. R. Lee (1986). "*Salmonella* in Swine at Slaughter: Incidence and Serovar Distribution at Different Seasons." Journal of Food Protection **49**(5): 366-368.

Curtiss, R. C., S. M. Kelly and J. O. Hassan (1993). "Live oral avirulent *Salmonella* vaccines." Vet. Microbiol **37**: 397-405.

Dahl, J., A. Wingstrand, F. Bager, D. L. Baggesen, B. Nielsen and K. Larsen (1996). Failure to eradicate *Salmonella typhimurium* by medication and strategic removal of pigs. 14th Congress Int Pig Vet Soc, Bologna, Italy.

Dahl, J., A. Wingstrand, D. L. Baggesen and B. Nielsen (1996). Eradication of *Salmonella typhimurium* by strategic removal of pigs in infected herds. 14th Congress Int Pig Vet Soc, Bologna, Italy.

Dahl, J., A. Wingstrand, D. L. Baggesen and B. Nielsen (1996). *Salmonella* reduction at the farm level. 14 Congress Int Pig Vet Soc, Bologna, Italy.

Dahl, J., A. Wingstrand, D. L. Baggesen and B. Nielsen (1996). Spread of *Salmonella* infection in pens and between pens. 14th Congress Int Pig Vet Soc, Bologna, Italy.

Dahl, J., A. Wingstrand, D. L. Baggesen, B. Nielsen and L. K. Thomsen (1996). The effect of a commercial organic acid preparation on seroprevalence and shedding of *Salmonella* in finishing pigs. 14th Congress Int Pig Vet Soc, Bologna, Italy.

Davies, P. R., W. E. M. Morrow, F. T. Jones, J. Deen, P. J. Fedorka-Cray and J. T. Gray (1997). "Risk of shedding *Salmonella* organisms by market-age hogs in a barn with open flush gutters." J Am Vet Med Assoc **210**(3): 386-389.

Dritz, S. S., K. Q. Owen, R. D. Goodband, J. L. Nelssen, M. D. Tokach, M. M. Chengappa and F. Blecha (1996). "Influence of lipopolysaccharide-induced immune challenge and diet complexity on growth performance and acute-phase protein production in segregated early-weaned pigs." J Anim Sci **74**(7): 1620-1628.

Dubey, J. P., P. Thulliez, R. M. Weigel, C. D. Williams, P. Linds and E. C. Powell (1995). "Sensitivity and specificity of various serologic tests for detection of *Toxoplasma gondii* infection in naturally infected sows." Am J Vet Res **56**(8): 1030-1036.

Duhamel, G. E., P. J. Fedorka-Cray, R. J. Bernard and E. D. Erickson (1993). Mice are involved in persistence of Salmonellosis on pig farms. Lincoln, NE, University of Nebraska.

Fedorka-Cray, P. J. (1993). Management Practices to Reduce Salmonellosis in Swine. 97 Ann Mtg United States' Animal Health Association.

Fedorka-Cray, P. J., E. Bush, L. A. Thomas and J. T. Gray (1996). Results of the 1995 NAHMS swine grower/finisher survey. 100 Ann. Mtg. USAHA, Little Rock, AR.

Fedorka-Cray, P. J., E. Bush, L. A. Thomas, J. T. Gray, J. McKean, D. L. Harris and G. Beran (1996). *Salmonella* infections in herds of swine. Ann. Mtg., Little Rock, AR.

Fedorka-Cray, P. J., J. T. Gray and L. A. Thomas (1995). Comparison of culture media for the isolation of *Salmonella* species from swine feces. Ames, IA, Iowa State University Extension.

Fedorka-Cray, P. J., L. C. Kelley, T. J. Stabel, J. T. Gray and J. A. Laufer (1995). "Alternate routes of invasion may affect pathogenesis of *Salmonella typhimurium*." Infection and Immunity **63**(7): 2658-2664.

Fedorka-Cray, P. J. and T. J. Stabel (1993). Influence of route of inoculation and dose on shedding and tissue distribution following infection of swine with *Salmonella typhimurium*. Ames, Iowa, Iowa State University.

Fernandez, L. R., G. E. Duhamel, B. E. Straw and P. J. Fedorka-Cray (1993). Diarrhea and rectal strictures on a commercial swine farm with enzootic salmonellosis caused by *Salmonella agona*. Annual Meeting American Association of Swine Practitioners, Kansas City, MO.

Ferris, K. E. and D. A. Miller (1996). *Salmonella* serotypes from animals and related sources reported during July 1995 - June 1996. 100 Ann. Mtg. USAHA, Little Rock, AR.

Galton, M. M., W. V. Smith, H. B. McElrath and A. B. Hardy (1954). "*Salmonella* in swine, cattle and the environment of abattoirs." J. Inf. Dis **95**(236-245).

Garcia, R. G. F., E. B. d. Oliveira, J. X. Viana, A. M. d. S. Filho and D. C. França (1978). "Sorotipos de *Salmonella* em matadouros-frigorificos. II Presença em gânglios linfáticos mesentéricos de suinos." Arq Biol Tecnol **21**: 123-127.

Glock, R. D. and D. L. Harris (1993). Disease management strategies: treat, control, or eliminate? Iowa State University Swine Disease Conference for Swine Practitioners, Ames, IA, Iowa State University Press.

Glock, R. D. and K. J. Schwartz (1993). Salmonellosis: A growing problem for food animal producers. Large Animal Veterinarian: 12-15.

Gray, J. T., T. J. Stabel and P. J. Fedorka-Cray (1996). "Effect of dose on the immune response and persistence of *Salmonella choleraesuis* infection in swine." Am J Vet Res **57**(3): 313-319.

Greenberg, B., G. Varela, A. Bornstein and H. Hernandez (1963). "*Salmonellae* from flies in a Mexican slaughterhouse." Am. J. Hyg **77**: 177-183.

Guthrie, R. (1992). *Salmonella*. Boca Raton, FL, CRC Press.

Hansen, R., R. Rogers, S. Emge and N. J. Jacobs (1964). "Incidence of *Salmonella* in the hog colon as affected by handling practices prior to slaughter." J Am Vet Med Assoc **145**(2): 139-140.

Harris, D. L. (1988). "Alternative approaches to eliminating endemic diseases and improving performance of pigs." Vet Rec **123**: 422-423.

Harris, D. L. (1992). Multiple isolated site production. 12th Cong Int Pig Vet Soc, Hague, Netherlands.

Harris, D. L. (1992). Multiple site production. Southeast Veterinary Conference, Raleigh, NC.

Harris, D. L., S. L. Edgerton and E. R. Wilson (1990). Large thymus glands in Isowean™ pigs. 11th Congress IPVS, Lausanne, Switzerland.

Harris, I. T., P. J. Fedorka-Cray, J. T. Gray, L. A. Thomas and K. Ferris (1997). "Prevalence of *Salmonella* organisms in swine feed." J Am Vet Med Assoc **210**(3): 382-385.

Hartwig, N. R. and D. O. Jones (1976). "Survey for *Salmonellae* in porcine bile and cecums and on equipment surfaces in an Ohio abattoir." JAVMA **169**(11): 1229-1230.

Hassan, J. O. and R. Curtiss (1994). "Development and evaluation of an experimental vaccination program using a live avirulent *Salmonella typhimurium* strain to protect immunized chickens against challenge with homologous and heterologous *Salmonella* serotypes." Infection and Immunity **62**(12): 5519-5527.

Heard, T. W., N. E. Jennett and A. H. Linton (1968). "The Control and eradication of Salmonellosis in a Closed Pig Herd." The Veterinary Record: 92-99.

Heard, T. W. and A. H. Linton (1966). "An epidemiological study of *Salmonella* in a closed pig herd." Journal of Hygiene (Cambridge) **64**: 411-417.

Henken, A. M., K. Frankena, J. O. Goelema, E. A. M. Gratt and J. P. T. M. Noordhuizen (1992). "Multivariate Epidemiological Approach to Salmonellosis in broiler Breeder Flocks." Poultry Science **71**: 838-843.

Heutgen, E. v., M. T. Coffey and J. W. Spears (1996). "Effects of immune challenge, dietary energy density, and source of energy on performance and immunity in weanling pigs." J An Sci **74**(10): 2431-2440.

Isa, J. M., B. R. Boycott and E. Broughton (1963). "A survey of *Salmonella* contamination in animal feeds and feed constituents." Canadian Veterinary Journal **4**(2): 41-43.

J.Garcia-Sirera, D. W. Rozenboom, B. Straw and P. Cray (1996). Survival of *Salmonella choleraesuis* in swine mortality during composting. 14th IPVS Congress, Bologna, Italy.

Jay, J. M. (1992). Modern food microbiology. New York, NY, Chapman and Hall.

Kampelmacher, E. H., P. A. M. Guinée, K. Hofstra and A. v. Keulen (1963). "Further studies on *Salmonella* in slaughterhouses and in normal slaughter pigs." Zentbl. Vet Med **10**: 1-27.

Kapperud, G. (1994). *Yersinia enterocolitica* infection. Handbook of Zoonoses. Section A Bacterial, Ricketssial, Chlamydial, and Mycotic. E.-i. -. C. G. W. Beran. Boca Raton, FL, CRC Press. **1**: 343-353.

Kapperud, G., S. M. Ostroff, T. Nesbukken, L. C. Hutwagner, N. H. Bean, J. Lassen and R. V. Tauxe (1994). Risk factors for sporadic *Yersinia enterocolitica* infections in Norway: A case-control study. 6th Int Symp on Yersinia, Rome, Italy, S. Karger AG.

Kliebenstein, J., J. J. Zimmerman, S. Patton and T. Roberts (1995). Prevalence of *Toxoplasma gondii* antibodies in hogs, farm management practice relationships, and economic costs. Ames, IA, Iowa State University Extension.

Konrad, H., B. P. Smith, G. W. Dilling and J. K. Kruse (1994). "Production of *Salmonella* serogroup D (O9)-specific enzyme-linked immunosorbent assay antigen." American Journal of Veterinary Research **55**(12): 1647-1650.

Kotula, A. W. and A. K. Sharar (1993). "Presence of *Yersinia enterocolitica* serotype O:5,27 in slaughter pigs." J. Food Protect. **56**(3): 215-218.

Kramer, T. T. (1995). "Swine Salmonellosis: What is New About it?" Agri-Practice **16**(1): 13-16.

Kramer, T. T., J. Rhiner and G. W. Beran (1995). *Salmonella* carrier detection at slaughter. Ames, IA, Iowa State University Extension.

Kramer, T. T., M. B. Roof and R. Matheson (1992). "Safety and efficacy of an attenuated strain of *Salmonella choleraesuis* for vaccination of swine." American Journal of Veterinary Research **53**(4): 444-448.

Lax, A. J., P. A. Barrow, P. W. Jones and T. S. Wallis (1995). "Current perspectives in salmonellosis." British Veterinary Journal **151**: 351-377.

Lee, J. A., A. C. Ghosh, P. G. Mann and G. H. Tee (1972). "*Salmonella*s on pig farms and in abattoirs." J. Hyg., Camb. **70**: 141-10.

Lind, P. (1995). ELISA for the detection of antibody produced against *Trichinella spiralis* in swine.

Lindsay, D. S., B. L. Blagburn and B. P. Stuart (1992). Coccidia and other protozoa. Diseases of Swine. A. D. Leman, B. E. Straw, W. L. Mengling, S. D'Allaire and D. J. Taylor. Ames, IA, Iowa State University: 661-667.

Linton, A. H. and T. W. Heard (1970). "Computer-based Analysis of Epidemiological Data Arising from Salmonellosis in Pigs." Research in Veterinary Science **11**: 523-532.

Lumsden, J. S. and B. N. Wilkie (1992). "Immune response of pigs to parenteral vaccination with an aromatic-dependent mutant of *Salmonella typhimurium*." Can J Vet Res **56**: 296-302.

Malmqvist, M., K.-G. Jacobsson, P. Häggblom, F. Cerenius, L. Sjöland and A. Gunnarsoon (1995). "*Salmonella* isolated from animals and feedstuffs in Sweden during 1988-1992." Act vet. Scand. **36**: 21-39.

Martin, S. W., A. H. Meek and P. Willeberg (1987). Veterinary Epidemiology: principles and methods. Ames, IA, Iowa State University Press.

McKinley, G. A., D. J. Fagerberg, C. L. Quarles, B. A. George, D. E. Wagner and L. D. Rollins (1980). "Incidence of *Salmonellae* in fecal samples of production swine and swine at slaughter plants in the United States in 1978." Appl Environ Microbiol **40**(3): 562-566.

Mills, K. W. and B. L. Kelly (1986). "Antibiotic susceptibilities of swine *Salmonella* isolants from 1979 to 1983." American Journal of Veterinary Research **47**(11): 2349-2350.

Morehouse, L. G. and E. E. Weman (1961). "*Salmonella* and other disease-producing organisms in animal by-products - A survey." Journal of the American Veterinary Medical Association **139**(9): 989-995.

Morse, E. V. (1974). "Salmonellosis - An environmental Health Problem." Journal of the American Veterinary Medical Association **165**(11): 1015-1019.

Morse, E. V., D. A. Milda and B. H. Blessman (1982). Survival of *Salmonella sp.* under natural simulated conditions in the swine environment. 25th Annual Meeting American Association of Veterinary Laboratory Diagnosticians.

Mousing, J., P. T. Jensen, C. Halgaard and J. P. Nielsen (1996). Concepts of the integrated *Salmonella* enterica control programme in Danish pork. 14th IPVS Congress, Bologna, Italy.

Nicholas, R. A. J. and G. A. Cullen (1991). "Development and application of an ELISA for detecting antibodies to *Salmonella enteritidis* in chicken flocks." Vet Rec **128**: 74-76.

Nielsen, B. and F. Bager (1995). Improving product quality. Diseases Conference, Ames, Iowa.

Nielsen, B., F. Bager, J. Mousing, J. Dahl, C. Halgaard and H. Christensen (1995). Danish perspective on the implementation of HACCP in the Swine Industry. Hazard Analysis and Critical Control Point (HACCP) Symposium, Chicago, Il, Conference of Research Workers in Animal Diseases.

Nielsen, B., D. Baggesen, F. Bager, J. Haugegaard and P. Lind (1995). "The serological response to *Salmonella* serovars typhimurium and infantis in experimentally infected pigs. The time course followed with an indirect anti-LPS ELISA and bacteriological examinations." Vet Microbiol **47**: 205-218.

Nielsen, B., D. Baggesen, F. Bager and P. Lind (1994). Serological diagnosis of *Salmonella* Infections in Swine By ELISA. 13th International Pig Veterinary Congress, Bangkok, Thailand.

Nielsen, B., D. L. Baggesen, P. Lind, N. Feld and A. Wingstrand (1996). Serological surveillance of *Salmonella* infections in swine herds by use of an indirect LPS ELISA. 14 Int Pig Vet Soc, Bologna, Italy.

Nielsen, B., D. L. Baggesen, P. Lind and A. Wingstrand (1995). Serological surveillance of *Salmonella* infections in swine herds by use of an indirect LPS ELISA. 4th International Veterinary Immunology Symposium, Davis, California, University of California.

Nielsen, B., J. Christensen, J. Mousing and P. T. Jensen (1995). Danish experience in swine serological profiling techniques. Diseases Conference, Ames, Iowa, Iowa State University Press.

Nielsen, B., C. Heisel and A. Wingstrand (1996). "Time course of the serological response to *Yersinia enterocolitica* O:3 in experimentally infected pigs." Vet Microbiol **58**: 293-303.

Nielsen, B., A. Wingstrand and C. Heisel (1996). *Yersinia enterocolitica* O:3 infection in Danish swine herds. 14 Congress Int Pig Vet Soc, Bologna, Italy.

Nielsen, J. P., C. Halgaard, P. Willeberg and J. Mousing (1996). *Salmonella* surveillance and control in Danish finishing herds. 14th IPVS Congress, Bologna, Italy.

Patterson, J. T. (1972). "*Salmonellae* in animal feedingstuffs." Record of Agricultural Research (Belfast) **20**: 27-33.

Reed, W. M., H. J. Olander and H. L. Thacker (1985). "Studies on the pathogenesis of *Salmonella heidelberg* infection in weanling pigs." Am J Vet Res **46**: 2300-2310.

Reed, W. M., H. J. Olander and H. L. Thacker (1986). "Studies on the pathogenesis of *Salmonella typhimurium* and *Salmonella choleraesuis* var *kunzendorf* infection in weanling pigs." Am J Vet Res **47**(1): 75-83.

Renwick, S. A., R. J. Irwin, R. C. Clarke, W. B. McNab, C. Poppe and S. A. McEwen (1992). "Epidemiological associations between characteristics of registered broiler flocks in Canada and the *Salmonella* culture status of floor litter and drinking water." Canadian Veterinary Journal **33**: 449-458.

Roof, M. B. and D. D. Doitchinoff (1995). "Safety, efficacy, and duration of immunity induced in swine by use of an avirulent live *Salmonella choleraesuis*-containing vaccine." American Journal of Veterinary Research **56**(1): 39-44.

Roof, M. B., T. T. Kramer, J. P. Kunesh and J. A. Roth (1992). "In vivo isolation of *Salmonella choleraesuis* from porcine neutrophils." Am J Vet Res **53**(8): 1333-1336.

SAS (1985). SAS user's guide: statistics. Cary, NC, SAS Inst. Inc.

Scheidt, A. B., T. R. Cline, L. K. Clark, V. B. Mayrose, W. G. V. Alstine, M. A. Diekman and W. L. Singleton (1995). "The effect of all-in-all-out growing-finishing on the health of pigs." Swine Health and Production 3(5): 202-205.

Schwartz, K. J. (1990). Salmonellosis in Midwestern swine. 94th Annual Meeting of the United States Animal Health Association, Denver, CO.

Smith, H. W. (1960). "The effect of feeding pigs on food naturally contaminated with *Salmonellae*." Journal of Hygiene (Cambridge) **58**: 381-389.

SPSS (1997). SPSS Base 7.5 for Windows® User's Guide. Chicago, IL, SPSS Inc.

Tay, S. C. K., R. A. Robinson and M. M. Pullen (1989). "*Salmonella* in the mesenteric lymph nodes and cecal contents of slaughtered sows." Journal of Food Protection **52**(3): 202-203.

Taylor, D. J. (1992). Infection with *Yersinia*. Diseases of Swine. A. D. Leman, B. E. Straw, W. L. Mengling, S. D'Allaire and D. J. Taylor. Ames, IA, Iowa State University Press: 639-640.

Thrusfield, M. (1995). Veterinary Epidemiology. Oxford, OX2 0EL, Blackwell Science, LTD.

USDA (1996). "Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) Systems; Final Rule." Federal Register **61**(144): 38805-38989.

vanSchie, F. W. and G. H. A. Overgoor (1987). "An analysis of the possible effects of different feed upon the excretion of *Salmonella* bacteria in clinically normal groups of fattening pigs." The Veterinary Quarterly **9**(2): 185-188.

Veldman, A., H. A. Vahl, G. J. Borggreve and D. C. Fuller (1995). "A survey of the incidence of *Salmonella* species and Enterobacteriaceae in poultry feeds and feed components." Veterinary Record **136**: 169-172.

Wilcock, B. and H. Olander (1978). "Influence of Oral Antibiotic Feeding on the Duration and Severity of Clinical Disease, Growth Performance, and Pattern of Shedding in Swine Inoculated with *Salmonella typhimurium*." Journal of the American Veterinary Medical Association **172**(4): 472-477.

Wilcock, B. P. and K. J. Schwartz (1992). Salmonellosis. Diseases of Swine. A. D. Leman. Ames, IA, ISU Press: 570-583.

Williams, B. M. (1975). "Environmental considerations in salmonellosis." Veterinary Record **96**: 318-321.

Wingstrand, A., C. Heisel, D. L. Baggesen, R. Hoff-Jørgensen, B. Nielsen, L. K. Thomsen and B. Bartholin (1996). Bovivac vaccine against experimental clinical *Salmonella typhimurium* infection in pigs. 14 Congress Int Pig Vet Soc, Bologna, Italy.

Wingstrand, A. and B. Nielsen (1996). Cross-sectional investigation of pig herds for *Yersinia enterocolitica* O:3. 14 Congress Int Pig Vet Soc, Bologna, Italy.

Wood, R. L., A. Pospischil and R. Rose (1989). "Distribution of persistent *Salmonella typhimurium* infection in internal organs of swine." Am J Vet Res **50**(7): 1015-1021.

Wood, R. L. and R. Rose (1992). "Populations of *Salmonella typhimurium* in internal organs of experimentally infected carrier swine." Am J Vet Res **53**(5): 653-658.

Wood, R. L., R. Rose, N. E. Coe and K. E. Ferris (1991). "Experimental establishment of persistent infection in swine with a zoonotic strain of *Salmonella newport*." Am J Vet Res **52**(6): 813-819.

Zimmerman, J. (1994). Toward an understanding of toxoplasmosis in swine. 8th International Congress on Animal Hygiene, St. Paul, MN, University of Minnesota and Washington State University.

Zimmerman, J. J., D. W. Dreesen, W. J. Owen and G. W. Beran (1990). "Prevalence of toxoplasmosis in swine in Iowa." J Am Vet Med Assoc **196**: 266-270.

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